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Climate-Induced Habitat Fragmentation Affects Metapopulation Structure of Arctic Grayling in Tundra Streams

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Climate change is altering ecosystems across the globe, with complex and varied ecological and evolutionary consequences that might affect species persistence. Here, I investigated the effects of climate-induced aquatic habitat fragmentation by river dry zones on Arctic grayling (*Thymallus arcticus*) metapopulation structure, microgeographic differentiation, movement patterns and vital rates. I used an integrative approach, combining neutral genetic microsatellite markers, remote sensing of PIT-tagged individuals, body condition and ovarian histology to examine evolutionary consequences of aquatic habitat fragmentation for Arctic grayling. I found that within my study area on Alaska's North Slope, Arcitc grayling comprised at least five distinct genetic clusters. River distance and dry zones were significant factors explaining genetic differentiation among locations. Estimates of effective population size suggested one large coastal population flanked by four smaller semi-isolated headwater populations. Migration was low and asymmetrical among genetic clusters, but higher from headwater populations to the large coastal population than contrariwise. Microsatellite markers revealed strong patters of microgeographic neutral genetic differentiation for larval Arctic grayling for two distinct populations. Adult Arctic grayling spawning movement patterns strongly associated with microgeographic neutral genetic differentiation. Although no significant differences existed in the spring between detained and non-detained individuals with regard to condition, fecundity, gonad phase or spring movement patterns following drought, I found significant differences in the following fall's movement patterns and differences in survival rates among detained and non-detained fish both post-spawning and in subsequent years. The association between dry zones and neutral genetic differentiation suggests that with climate change, small headwater populations might become increasingly isolated, which could increase probability of local extirpations. Additionally, selection against spawning locations with high summer

drying frequency might isolate spawning activities and reduce gene flow among spawning locations. Furthermore, detainment by dry zones appears to alter demographic rates by decreasing adult survival and reducing the number of potential spawning events for this long-lived iteroparous species.

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Dedication

This work is dedicated to my mother, Ann Harlow Geyer Zimmerman, who unabashedly bragged about her daughter, the scientist. I wish you could be here to see this dissertation come to fruition. I know you would be proud.

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Introduction

The Arctic is undergoing some of the most rapid and severe climate-driven environmental changes on the planet (IPCC 2013; ACIA 2004), with Alaska's North Slope experiencing an upward trend in annual mean surface air temperature. Since 1960, the balance between summer precipitation and evapotranspiration rates has shifted, leading to an increased water deficit for this region of 5.5 mm/year (Larry D. Hinzman et al. 2005a). This changing summer water budget alters stream discharge (Kane et al. 2004), which along with other climate-driven hydrologic changes (Brosten et al. 2006; Zarnetske et al. 2008) causes streams in the region to increase frequency and duration of river drying (Erica D Betts & Kane 2015). Habitat fragmentation from river drying can affect species within the aquatic landscape by altering spatially dynamic metapopulation processes, such as colonization and extirpation of habitat patches, and by changing access to critical habitats within local population ranges, including access to food, refugia and spawning locations (Opdam & Wascher 2004). Yet with increasing vulnerability of the North Slope's aquatic habitats to fragmentation from river drying, we currently do not understand basic population structure for many species, let alone the degree to which habitat fragmentation and local conditions influence dispersal and persistence of aquatic organisms.

Increased fragmentation of Arctic streams could hierarchically influence stream populations, from metapopulation structure among populations, to microgeographic differentiation within populations, to vital rates of local populations, and therefore necessitates a multifaceted research approach. Assessing metapopulation structure allows investigation of broad-scale habitat connectivity among populations, including dispersal distance, physical barriers, resistance features, and even population size. On a smaller scale, within species dispersal range, investigation of traits that might be influenced by aquatic fragmentation, such as seasonal migration patterns, allows insight into species adaptability to changing conditions. Examining the affects of fragmentation on population vital rates (births and deaths) allows assessment of population viability under climate change. In this dissertation, I investigate aquatic habitat

fragmentation by river drying on multiple spatial scales employing a metapopulation framework, which explores metapopulation structure, microgeographic differentiation and population demographics.

Chapter 1: Understanding factors influencing species metapopulation structure is critical because relationships within and among local populations determine the relative contributions of gene flow, drift, and adaptive potential for the species. Habitat connectivity facilitates dispersal of individuals among local populations, which can either increase or decrease genetic variability depending on the balance between gene flow, drift, and local selection. Maintenance of genetic variability is essentail for species adaptability, but while some degree of fragmentation might increase overall genetic variability, high levels of fragmentation might lead to local population isolation, decreased effective population size and loss of genetic diversity within local populations (Hanski 2011). For example, for cutthroat trout on the Oregon coast, stream fragmentation led to population isolation, reducing gene diversity and causing significant genetic structure both among and within tributaries (Wofford 2005). Nevertheless, Kapralova et al. (2011) found that contemporary and recent patterns of restricted gene flow facilitated local adaptive evolution and maintenance of adaptive genetic variation in Icelandic Arctic charr. Thus, climate induced changes in habitat connectivity might influence dispersal rates, altering the extent of population isolation and ultimately determining the degree of genetic variability among populations (Hanski & Mononen 2011; Hanski et al. 2011).

I investigate factors influencing metapopulation structure for Arctic grayling across a broad geographic scale. Because we currently lack basic knowledge regarding Arctic grayling population genetic structure, I explore what constitutes a population, what is the species' potential dispersal capability, and what factors influence dispersal among local populations. Answers to these questions are critical for any effective conservation or management effort. I predicted that river distance, elevation, watershed boundaries, and habitat-fragmenting ephemeral dry river zones would increase

genetic differentiation, creating spatially distinct neutral genetic patterns by restricting gene flow among local populations.

Chapter 2: Climate-induced aquatic habitat fragmentation might also affect genetic variability within local populations by providing variation in habitability of river segments, thereby imposing strong selection upon genotypes that express variation in selectively susceptible phenotype. This type of microgeographic genetic differentiation can occur when populations adapt differentially at spatial scales within dispersal distance of the species (Richardson & Urban 2013). The response of a species to environmental change depends not only upon the magnitude, direction and timing of change, but upon species plasticity and/or adaptability to new local conditions (Fitzpatrick 2012). In the absence of physical barriers, gene flow might nevertheless be restricted by selective factors, such as differential predation regimes (Richardson & Urban 2013), spawning site fidelity (Garcia de Leaniz et al. 2007) or mate preference (Maan et al. 2004). Dry river zones might provide differential selection regimes across space, yet within dispersal range of the species, thereby imposing strong selection for different phenotypes that ultimately limit gene flow.

I test for association between microgeographic genetic differentiation and an adult trait, spawning migration distance. Here, I narrow my spatial scope to two genetically distinct populations: the Kuparuk River and Oksrukuyik Creek. In particular, I test for microgeographic differentiation of Arctic grayling in these streams using larval fish as a substitute for spawning stocks, and investigate spawning migration distance with regard to fine-scale neutral genetic differentiation using microsatellite markers and remote sensing of tagged adults. I predicted that adult Arctic grayling would express variation in migration distance from overwintering locations; that larval grayling would express microgeographic genetic differentiation; and that adult migration distance and genetic signatures would correlate positively with site-specific larval microgeographic genetic differentiation.

Chapter 3: Habitat fragmentation might affect population vital rates by changing synchronization between resource acquisition and growth, survival and reproduction (Marchand 1996; Ganias et al. 2011). In the Arctic, in order to optimize resource acquisition, timing of movement patterns must maximize access to resources, while avoiding freezing in winter (Power & Reynolds 1997). For fish, environmental conditions leading up to spawning could affect individual condition, which might determine spawning capability, as was found for anchovy (Pecquerie et al. 2009), herring (Pangle et al. 2004) and bluegill sunfish (Cargnelli & Neff 2006). River drying, therefore, might affect population vital rates through reduced habitat quality when migratory fish are detained in streams due to habitat fragmentation. Within population connectivity might prove critical for controlling local population birth and death rates, which in turn influence population viability and subsequently population extirpation rate (Hanski 2011).

I opportunistically investigate the effects of drought on vital rates and movement patterns of Arctic grayling in the Kuparuk River. Focusing on a single population, I compare demographic differences and movement patterns between fish detained by drought and fish that avoided detainment. I predicted that detained fish would show increased rates of oocyte atresia (egg resorption), alter spring movement patterns by skipping spawning, and show decreased post-overwinter survival and condition compared to non-detained individuals.

Using a metapopulation framework, which explores metapopulation structure, microgeographic differentiation and population demographics, this study seeks to assess potential impacts of climate-induced changes in aquatic habitat connectivity for Arctic grayling on Alaska's North Slope. Employing an integrative approach combining neutral genetic microsatellite markers, remote sensing of PIT-tagged individuals, body condition and ovarian histology, I examine evolutionary consequences of aquatic habitat fragmentation in a system undergoing rapid climate change. With insights gained through this research, I aim to provide examples applicable to other species that will enhance predictive approaches to

management and conservation based on metapopulation theory and ultimately mitigate affects of climate change and habitat fragmentation on species persistence.

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Chapter 1:

Connectivity structures genetic divergence of an Arctic freshwater fish metapopulation

Abstract

Climate change will likely alter habitat connectivity for many organisms. Connectivity, in turn, can fundamentally influence metapopulations by either facilitating or reducing movement among local populations. I examined the influence of multiple factors contributing to habitat connectivity on genetic population structure, recent migration rates and effective population size for Arctic grayling on Alaska's North Slope. I predicted that isolation by river distance, ephemeral river dry zones, elevation and watershed boundaries would influence genetic differentiation and population structure. Analysis of 437 individuals from 16 geographic locations using 10 microsatellite loci revealed significant population genetic structure (F_{ST} from 0.028 to 0.064). Using both Bayesian assignment and discriminant analysis of principle components (DAPC), I identified five genetically distinct clusters. Mantel and Partial Mantel tests indicated that river distance, watershed boundaries and ephemerally dry river zones were significantly associated with genetic differentiation among sampled locations. Effective population size indicated a large "mainland" population west of the Kuparuk River and smaller "island" populations within the Kuparuk and Sagavanirktok watersheds. Bidirectional migration rates signified asymmetric dispersal among locations with higher rates of dispersal from island populations to the mainland than from the mainland to island populations. This study suggests that ephemeral environmental features influence genetic diversity and population structure and stresses the importance of maintaining habitat connectivity for metapopulation persistence and species management and conservation.

Introduction

Connectivity among populations is fundamental to the genetic structure, ecology, and persistence of metapopulations and thus is a central focus of ecology, evolution, and conservation biology (Hanski &

Gilpin 1997). Factors influencing connectivity affect movement of individuals by either allowing or restricting access to available habitat (Orsini et al. 2013; Fagan 2002). Changes to the environment that result in habitat fragmentation or resistance to movement can alter dispersal among local populations, influencing population dynamics, genetic diversity through gene flow, and metapopulation structure over time (Hanski 1989; Hanski 2011; Whitlock 2004; Morrissey & Kerckhove 2009; Pauls et al. 2013). In an era of rapid climate change where species must either migrate or adapt to new environmental conditions, understanding population structure, connectivity and the environmental factors associated with genetic characteristics of populations can allow for more effective species management and conservation (Stockwell et al. 2003; Matala et al. 2014; Hanski 2011; Hansen et al. 2008; Rieman & Dunham 2000).

Because freshwater fish are constrained to the paths of rivers and lakes, these species might be particularly sensitive to disruptions in connectivity among local populations (Fagan 2002; Fluker et al. 2014; Leclerc et al. 2008; Perkin et al. 2014). Anthropogenic barriers, such as dams and culverts, are often implicated as factors restricting dispersal (Junker et al. 2012; Peterson & Ardren 2009; Roberts et al. 2013). Natural environmental drivers, such as droughts and floods (Fitzpatrick et al. 2014; Meeuwig et al. 2010; Hopken et al. 2013; Perkin et al. 2014), or location specific life-history factors, such as spawning areas and overwintering locations (Ozerov et al. 2012; Vähä et al. 2007; Fausch et al. 2002; Maria et al. 2012), influence evolutionary processes in dendritic systems, as well. Changes in hydrology that alter freshwater habitat connectivity have been shown to influence genetic structure of aquatic species (Mossop et al. 2015; Fagan et al. 2007; Poissant et al. 2005). Understanding and conserving key metapopulation processes, including spatial structuring and factors that influence dispersal, colonization potential and extirpation risk are likely to vary with life history, species, scale, and landscape (Rieman & Dunham 2000).

Climate driven changes in stream hydrology in the Arctic might be particularly significant in structuring freshwater populations that face high mortality due to detainment or inability to move into overwintering sites. In order to optimize use of stream habitat, Arctic fish must time movements to maximize access to stream resources, while avoiding freezing in winter (Power & Reynolds 1997). On

Alaska's North Slope, for example, rivers provide spawning and feeding habitat for Arctic grayling during the spring and summer, yet dry river zones, occur periodically and can greatly alter aquatic connectivity (Betts & Kane 2015), thereby stranding fish within the river. Increasing temperature due to polar amplification of climate change portends increased duration and extent of river drying (ACIA 2005; Hinzman et al. 2005; Martin et al. 2009). Summer surface water balance shifts negative as temperature driven uptake by plants exceeds summer precipitation (Hinzman et al. 2005). Consequently, water available to maintain river flow diminishes, leading to reduced flow rates and increased river drying (Betts & Kane 2015). Increased frequency of freshwater habitat fragmentation due to river drying could alter species movement patterns and dispersal ability, particularly if drying occurs during key migratory timeframes. As such, fragmenting of Arctic freshwater systems by river drying could restrict species movement and dispersal, ultimately leading to the formation of small, reproductively isolated populations (Junker et al. 2012; Primmer et al. 2006). Climate induced changes in aquatic habitat connectivity, therefore, could affect local population genetic diversity and metapopulation structure and persistence (Reist et al. 2010). Yet, with research mainly focused on ecological interactions of Arctic freshwater fish in the Toolik Lake area of the North Slope, AK (Deegan et al. 1997; Deegan et al. 2005; Deegan et al. 1999; Buzby & Deegan 2000; Slavik et al. 2004; Deegan & Peterson 1992; Hershey et al. 2006), we still do not fully understand how populations are distributed across the aquatic landscape, what factors influence dispersal among populations and the evolutionary consequences of changing connectivity.

I hypothesized that landscape and environmental factors influencing aquatic connectivity would create spatially distinct neutral genetic patterns for Arctic grayling. In particular, I predicted that historic factors, such as river distance, elevation and watershed boundaries, as well as an ephemerally occuring factor, dry river zones, would decrease genetic differentiation by restricting gene flow among local populations (Reilly et al. 2014; Hershey et al. 2006; Clarke et al. 2005; Mossop et al. 2015). Dispersal limitation within the stream network might lead to a pattern of isolation by river distance. Increasing elevation might be associated with genetic differentiation by limiting upstream dispersal, producing

patterns of asymmetric gene flow favoring downstream migration. Watershed boundaries might act as barriers to migration because freshwater fish must travel through estuarine water in order to enter a different watershed and therefore they might reflect historic colonization events from ancient glacial refugia. Ephemeral dry river zones might be associated with genetic differentiation by forming physical barriers to fish movement, thereby reducing gene flow among locations. Alternatively, Arctic grayling's long-distance dispersal ability and overlapping generations might override the effects of genetic drift, fostering a panmictic genetic distribution.

Materials and Methods

Natural history and site description

Arctic grayling traverses between lake and stream habitats through a dendritic aquatic matrix. Within my study area on Alaska's North Slope (Figure 1), Arctic grayling are prevalent within the three major watershed drainages, the Itkillik, Kuparuk and Sagavanirktok, which were formed during the middle to late Pleistocene (Hamilton 2003). As in other areas of its range, Arctic grayling on the North Slope exhibit different habitat and movement patterns depending on location, including fluvial (within streams), adfluvial (within lakes and streams) and lacustrine (non-migratory, remaining in lakes) movement patterns (Beauchamp 1990, West et al. 1992, Parkinson et al 1999). All viable populations, however, need to move to and from appropriate spawning, feeding and overwintering habitats, which might be over 100 km apart (West et al. 1992). Additionally, similar to the closely related European grayling, *T. thymallus*, (Mallet et al. 2000), larval, juvenile and adult Arctic grayling life stages often require distinct habitat patches. Thus, population persistence likely depends on the spatial and temporal connectivity of the freshwater environment (Opdam and Wascher 2004) and on Arctic grayling's potential for adaptation to changing conditions.

Fish Sampling

I sampled Adult Arctic grayling during the open water season (May to August) from 2010 to 2013. My sampling of the North Slope of Alaska included 15 locations around the Toolik Research Area: eight tundra streams and seven lakes. I also included one stream on the coastal plain to serve as an outgroup population (Figure 1, Table 1). Sampling locations were chosen to stratify habitats within and among watersheds and to represent locations separated by varying degrees of seasonal river drying. Arctic grayling were collected using a combination of seine, fyke, and gill nets, as well as via hook and line. I collected a caudal fin tissue sample from each individual and the tissue was preserved in 95% ethanol and stored at -20° C until DNA extractions were conducted.

Genotyping and descriptive statistics

DNA was extracted from fin tissue using DNeasy blood and tissue kits (Qiagen, CA). Multiplex PCR reactions were optimized for allelic range for twelve highly variable nuclear microsatellite markers specific to Arctic grayling (Diggs & Ardren 2008) in a manner similar to Steed (2007) (Table S1, supporting information). PCR products were analyzed on an ABI DNA sequencer and allele sizes were scored along with positive and negative controls using the program GeneMarker. All genotypes were hand-checked for accuracy. Amplifications that were too weak to resolve peaks or had excess stutter were re-amplified and rerun for better resolution. Any remaining unresolved alleles were treated as missing data.

I screened for null alleles, large allele dropout and scoring errors using the program MICRO-CHECKER v.2.2.3 (Van Oosterhout et al. 2004). Exact tests (Guo & Thompson 1992) were used to test for deviations from Hardy-Weinberg equilibrium across all loci and all populations with 1,000,000 Markov Chain Monte Carlo (MCMC) and 100,000 dememorization steps in the program ARLEQUIN v3.5 (Excoffier & Lischer 2010). I also used ARLEQUIN v3.5 to test for deviations from linkage disequilibrium across all pairs of loci using an expectation-maximization algorithm with 10,000 permutations. Probability values were Bonferroni corrected whenever multiple testing occurred.

Descriptive statistics, including allelic richness, private alleles and observed and expected heterozygosity, were calculated using GenoDive 2.0b23 (Meirmans & Van Tienderen 2004). Unbiased estimates of allelic richness and private alleles per sample location were calculated via rarefaction using the program *HP-Rare 1.0* (Kalinowski 2005). I examined patterns in heterozygosity and allelic richness with latitude using linear models and model selection based on Akaike information criterion (Bates et al. 2015).

Analysis of population structure

I calculated F_{ST}, G'_{ST} and Jost's D to summarize genetic differentiation and found that all metrics produced similar results. For simplicity, I only report *F*-statistics. Estimates of genetic differentiation (F_{ST}) and inbreeding coefficients (F_{IS} and F_{IT}) were obtained using the program ARLEQUIN v3.5. I examined pairwise genetic differentiation and its significance among sites using GenoDive 2.0b23 and GENEPOP (Rousset 2008) with the Markov Chain parameterized using 10,000 dememorization steps, 100 batches and 5,000 iterations per batch. Bi-directional pairwise migration rates (*m*) for all sampled locations were calculated using the program BAYESASS3 (Wilson & Rannala 2003b) with random seed and 20,000,000 MCMC iterations, which included a burn-in of 3,000,000 iterations and a sampling interval every 100 iterations. Model parameters were optimized by first adjusting mixing value acceptance rates, then using the program TRACER v1.6 (Rambaut et al. 2003) to diagnose convergence.

Population structure was inferred using complementary approaches: Bayesian assignment in STRUCTURE (Pritchard et al. 2000) and discriminant analysis of principle components using DAPC within the *Adegenet* package (Jombart et al. 2010) in R. STRUCTURE was used to estimate the number of genetic clusters (K) using the log likelihood of individual assignment into K inferred genetic clusters. I used a burn-in length of 25,000 iterations preceding each MCMC simulation (100,000 iterations for K = 1 to 12, repeated 20 times for each value of K). The program STRUCTURE HARVESTER (Earl & VonHoldt 2011) was used to assess and visualize likelihood values, including L(K), L'(K), L''(K) and

ΔK (Evanno et al. 2005), in order to detect the number of genetic clusters that best fit the data. The program CLUMPP (Jakobsson & Rosenberg 2007) was used to optimize STRUCTURE runs, and the program DISTRUCT (Rosenberg 2003) was used to visualize the final solution for the optimal number of genetic clusters. Furthermore, DAPC from the R package ADEGENET provided a complementary assessment of genetic structure, free from underlying assumptions regarding Hardy-Weinberg equilibrium or linkage disequilibrium. I also used DAPC to investigate hierarchical sub-structure within genetic clusters. I used information derived from multiple sources including STRUCTURE, DAPC, ARLEQUIN, GENEPOP and BA3 to determine a consensus value for K.

I examined differences among locations with regard to assignment probabilities to genetic clusters using permutation tests in R. I created random distributions of assignment probabilities derived from STRUCTURE output by randomly shuffling group labels (headwater versus downstream) for each watershed tested (Kuparuk and Oksrukuyik). I then resampled 9999 times to attain random distributions of mean differences in assignment probabilities. I then compared my observed mean difference in assignment probabilities to my random distribution. I obtained p-values using a two-tailed significance test in R.

Effective population size

I estimated effective population size (N_e) to compare relative numbers of individuals among sampled locations and among genetic clusters with the intent to (1) investigate the potential contribution of population size to migration estimates (Wang et al. 2003); and (2) infer influences of population size on local population persistence (Palstra & Ruzzante 2008). I used the linkage disequilibrium method (Waples & Do 2008) implemented in the program NeESTIMATOR (Do et al. 2014) and analyzed the data based on both sampled locations and genetic clusters. Evaluating N_e based on sampled locations allowed us to investigate geographic differences in N_e, while larger sample sizes based on genetic clusters provided better accuracy and precision of N_e estimates for each genetically defined group (England et al. 2006). Additionally, I selected N_e based on *Pcrit*, which excludes certain frequencies of rare alleles depending on

sample size, thereby reducing downward bias that occurs when sample size is low (Waples & Do 2010; England et al. 2006).

Recent pairwise migration rates

I calculated contemporary bi-directional migration rates to estimate gene flow over the last two generations using BAYESASS v3.0 (Wilson & Rannala 2003a). This Bayesian assignment method provides estimates of gene flow by inferring inbreeding coefficients, where immigrants and their progeny display genotypic disequilibrium relative to the population from where they were sampled. Although free from the assumptions of Hardy Weinberg Equilibrium, the method assumes that background migration rate is relatively low ($F_{ST} > 0.05$) and that loci are in linkage equilibrium. I obtained estimates of posterior mean migration rates and the standard deviation of the marginal posterior distribution using a random starting seed, MCMC chain of 10^6 iterations and a 10^5 iteration burn-in interval. I adjusted the migration, allele frequency and inbreeding coefficient mixing parameters to 0.5, 0.5, and 0.7, respectively, which ensured that the proposed changes between chains were between 40 and 60% of the total number of iterations (Rannala 2013). Convergence was assessed using TRACER v1.6 (Rambaut & Drummond 2009). I first ran the analysis for all 16 locations to examine dispersal patterns among locations. I then ran the analysis using the same individuals re-grouped into genetic clusters inferred from the DAPC analysis.

Isolation by distance and landscape environmental features

Patterns of isolation by distance occur when gene flow strongly correlates with geographic distance separating populations (Jenkins et al. 2010), resulting in a gradient of decreasing genetic similarities across the landscape. Isolation by distance predicts differentiation as a result of dispersal limitation and drift (Sexton et al. 2014). I tested for isolation by distance by performing Mantel tests of matrix correlation between the genetic distance matrix (F_{ST}) and pairwise river distance (km) measured between populations with 10,000 permutations in the VEGAN package in R (Oksanen et al. 2013). Mantel tests use permutations to account for non-independence of pairwise matrix elements in order to assess the

significance of correlations. I address criticism regarding the efficacy of Mantel and partial Mantel tests by testing a priori hypotheses using data consisting entirely of distance measures (Legendre & Fortin 2010; Kierepka & Ecology 2015) and testing for correlations among environmental resistance matrices prior to conducting formal analyses with partial Mantel tests (Diniz-Filho et al. 2013a).

I hypothesized that in addition to isolation by river distance, environmental variables among locations, including watershed boundaries, estuaries, elevation and river dry zones, act as resistance features to produce patterns of isolation by environment (Sexton et al. 2014), thereby increasing genetic differentiation among locations beyond that predicted by isolation by distance alone. I derived spatially explicit environmental covariates using the STARS ArcGIS toolset (Peterson & Ver Hoef 2014) in ARCMAP v10.2 (ESRI 2013). GIS data included a digital elevation model (SDMI 2013) and stream and water body shapefiles (USGS 2014). Location and number of dry river zones were assessed using GPS coordinates from helicopter flight surveys and ground-truthing, as well as with game cameras and/or temperature and pressure loggers placed at various locations throughout the study system. To test for co-linearity among environmental distance matrices, I tested for correlations using Mantel tests and reduced the factors based on significant correlations for Mantel's r (Pearson correlation) of 0.7 or greater. I reduced the hypotheses for inclusion in partial Mantel tests for isolation by environment to the following factors: extent of river drying (km), watersheds, and elevation (m) (Tables S1, S2 and S3; Supplemental Tables). Partial Mantel tests were conducted using 10,000 permutations in the R package VEGAN (Oksanen et al. 2013).

Results

Microsatellite Screening and summary statistics

Of 12 microsatellite loci originally employed, two loci (*Tar*109 and *Tar*112) showed homozygote excess in five out of 16 populations and showed evidence of either null alleles, large allele dropout, or scoring errors (Van Oosterhout et al. 2004) and, therefore, these two loci were removed from further analyses.

The final dataset included 16 sampled locations and 10 loci, and a total of 437 individuals sampled. Across the remaining 10 loci, I found no significant deviations from Hardy Weinberg equilibrium, except for a single locus (*Tar*114) in only one population (Oksrukuyik Creek) with p-value ≤ 0.0003. I found no evidence of linked loci after applying a Bonferroni correction. All remaining loci were highly polymorphic with 32.2 ± 6.3 (standard deviation) mean number of alleles per locus (Table S1, supporting information) and location specific gene diversity ranging from 0.8355 to 0.9338 (Table S2, supporting information). Number of alleles per sample location varied from 11 to 20 alleles with similar patterns of diversity reflected in effective number of alleles, rarified allelic richness, rarified private allele richness and heterozygosity (Table 1). I found no correlation between increasing heterozygosity and increasing latitude after removing the one outlying population, the Ublutuoch on the coastal plain, from the analysis. In four of five cases, private allele richness increased from 0.2 for headwater locations to 0.4 for downstream locations, with similar patterns of diversity for all indices tested (Table 1). The two locations situated West of the Kuparuk River, however, expressed relatively high allelic richness compared to all other locations and demonstrated an increasing pattern of diversity from headwaters (Itkillik) to coastal plain (Ublutuoch) (PrA_r= 1.3 and 1.0, respectively).

Number of genetic clusters

Results from STRUCTURE and DAPC indicated significant genetic structure among Arctic grayling populations across the study area, with genetic clusters associating with specific geographic areas. STRUCTURE produced a maximum log probability of the data starting at K = 5, with a substantial increase in standard deviation at and above K = 6 (Figure 2a). Using the Evanno *et al.* (2005) method, ΔK revealed peaks at K = 2, K = 5 and K = 7 (Figure S1). But, exceptionally high variance in Ln(K) at K= 6 greatly influenced calculations of ΔK beyond this point, likely leading to an exaggerated peak at $\Delta K = 7$. STRUCTURE found similarity among individuals from the Atigun/Sagavanirktok watershed (Figure 3a, pink), Oksrukuyik watershed (Figure 3a, yellow), the Kuparuk watershed (Figure 3a, green) and the Toolik watershed (Figure 3a, red) when I employed K = 5 genetic clusters. STRUCTURE also identified

strong similarity among individuals from the upper Itkillik River (IT) and Ublutuoch River (UB) on the coastal plane (Figure 3a, purple). Using DAPC, I found K = 5 genetic clusters, which were associated with similar geographic regions to those found in the STRUCTURE analysis: Atigun/Sagavanirktok, Oksrukuyik, Kuparuk, Toolik and Itkillik (Figure 5).

Analysis of molecular variance (AMOVA) yielded p-values < 0.001 for comparisons of global *F*-statistics, indicating significant genetic structure across the study area. *F*-statistics suggested relatively high genetic differentiation among individuals within the total population (F_{IT} = 0.057) and among subpopulations (F_{ST} = 0.041) compared to differentiation among individuals within subpopulations (F_{IS} = 0.017). Pairwise F_{ST} showed greater similarity within certain geographic locations, including the Atigun River drainage (AT, G1, TL and USag); between Oksrukuyik Creek (OC) and Campsite Lake (CS); along the upper Kuparuk River (GCL, KUS, UKup and L86); within the Toolik Lake area (T and S3); and among the Ublutuoch and Itkillik sites (UB and IT) (Table 2). These groupings based on pairwise F_{ST} values concurred with clustering results from *Structure* and DAPC. Additionally, analysis of pairwise migration rates (*m*; Table 5) showed high residency status (*m* > 0.8) for five of the 16 sampled locations, which where in agreement with findings from pairwise F_{st}, *Structure* and DAPC.

Population structure within watersheds

I analyzed the data for additional sub-structure within watersheds using STRUCTURE and DAPC. In the Kuparuk and Oksrukuyik watersheds, headwater locations differed from downstream locations (Figure 3b and c). I also analyzed subsets of the data based on five genetic clusters from DAPC and found that each cluster contained at least two sub-clusters based on Bayesian information criterion (BIC). STRUCTURE plots (Figure 3) and assignment probabilities derived from DAPC and plotted across the aquatic landscape (Figure 1) illustrate the degree to which each genetic cluster associated with geographic locations from which individuals were sampled. Differences in the geographic distribution of genetic clusters and assignments to those clusters showed association with ephemeral river dry zones (Figure 1,

red lines). Within the Kuparuk and Oksrukuyik watersheds, headwater locations had significantly higher proportions of individuals assigned to the dominant genetic cluster for that watershed compared to downstream locations, indicating mixing of genetically distinct individuals at downstream sites (GCL versus LKup: p-value < 0.0001; OC versus LSag: p-value < 0.0001).

Effective population size

Effective population size (N_e) was highest for the Itkillik genetic cluster (N_e = 5,819 individuals; CI 1043, infinite), an order of magnitude larger than the other four genetic clusters (N_e = 215 to 455 individuals with largely overlapping confidence intervals; Table 3; Figure 5). Estimates of effective population size based on sampled locations ranged from 53 individuals to an infinite number of individuals (Table 4). Low sample size compared to actual (unknown) effective size for these sites, however, likely influenced accuracy of the estimates for many locations (England et al. 2006).

Recent pairwise migration

Analysis of pairwise migration rates over the last two generations (*m*) provided evidence of asymmetric dispersal among genetic clusters (Table 5; Figure 5). Asymmetric dispersal from the Kuparuk, Oksrukuyik and Atigun genetic clusters to the Itkillik genetic cluster occurred at approximately the same rate and indicated low levels of gene flow from the headwaters to the coastal plain (*m* = 0.05 to 0.06). The strongest asymmetry occurred between the Kuparuk and Toolik genetic clusters, where migration occurred 80x more frequently than migration from the Kuparuk to the Toolik genetic cluster than from the Toolik to the Kuparuk genetic cluster.

In agreement with my prediction regarding dispersal direction, migration among sampled locations occurred more frequently in a downstream direction (71% downstream). Contrary to my prediction, however, I found no difference between migration rates for downstream and upstream movement (Kruskal-Wallis chi-squared = 0.0462, df = 1, p-value = 0.8298, for *m* > 0.01). Analyses

suggested that fish in the Atigun and Kuparuk watersheds demonstrated downstream dispersal; fish in the Oksrukuyik watershed displayed bidirectional dispersal; and fish in the Itkillik watershed favored upstream dispersal (Table 6).

Environmental correlates of structure

I found a highly significant pattern of isolation by river distance (Mantel's r = 0.7038; r^2 = 0.4953; P < 0.0001). Plotting pairwise genetic differentiation against pairwise river distance showed a pattern of increasing differentiation up to approximately 400 km (Figure 6), beyond which this relationship diminished suggesting that 400 km might serve as a rough estimate of dispersal distance for this species (Jaquiéry et al. 2011). Compared to predictions based on gene flow versus genetic drift (Koizumi et al. 2006), locations separated by a high extent of river drying (>15 km) exhibited a pattern dominated by genetic drift compared to populations separated by lesser extents of river drying (Figure 6).

Partial Mantel tests evaluate how two matrices are correlated after controlling for the effects of IBD, thereby evaluating the effects of isolation by environment. Partial Mantel tests demonstrated a significant association between standardized genetic divergence [$F_{ST}/(1-F_{ST})$] and extent of river drying (Mantel's r = 0.5298; r^2 = 0.2807; p < 0.0001), suggesting that 28% of the genetic variation remaining after accounting for isolation by distance can be explained by the extent of river drying among sampled locations. Major watersheds (Mantel's r = 0.1155; r^2 = 0.0133; p = 0.1480), elevation (Mantel's r = -0.2429; p = 0.9768), and estuary extent (Mantel's r = -0.1993; p = 0.9875) did not significantly contribute to the remaining genetic variation after accounting for isolation by distance.

Discussion

Consistent with my hypothesis, spatially distinct patterns of population genetics were strongly influenced by river distance and aquatic habitat fragmentation, such that genetic differentiation among populations was significantly higher for remote locations and where aquatic connectivity was low. Additionally, river dry zones were strongly associated with neutral genetic differentiation for Arctic grayling, suggesting that

they might act as barriers to moving. The results did not support elevation as a barrier to movement among locations. However, within watersheds, the frequency of downstream migration exceeded that of upstream migration. Comparable results from three analyses (genetic clustering, Bayesian inference of migration rates and effective population size) supported high levels of population structure for Arctic grayling with asymmetric dispersal and semi-isolated local populations. These inferences suggest that North Slope Arctic grayling form a type of mainland-island metapopulation in which dispersal rates from island populations to the mainland population exceeded those from the mainland to the islands.

Isolation by distance

I discovered significant isolation by distance concurrent with results found by other population genetic studies for Arctic grayling (Stamford & Taylor 2005; Stamford & Taylor 2004a; Reilly 2014). My study agrees with Reilly et al. (2014), such that Arctic grayling heterozygosity was highest on the coastal plain and lower for populations at higher latitudes located in the headwaters. This pattern might indicate dispersal from ancient refugia in the north and subsequent colonization of upstream headwater locations to the south. Increased genetic differentiation with river distance (isolation by distance) likely reflects dispersal of grayling from the North Beringia glacial refuge, approximately 10,000 years ago (Stamford & Taylor 2004) into newly available stream habitat following successive glaciations (Hamilton 2003). The pattern of isolation by distance in the data suggests a stepping-stone model of dispersal (Kimura & Weiss 1964), similar to that found for other stream dwelling salmonids (Koizumi et al. 2006; Garza et al. 2014; Barson et al. 2009). However, despite the Arctic grayling's ability to traverse the aquatic landscape (West et al. 1992), I found genetic differentiation at fine spatial scales also similar to other salmonid species (Poissant et al. 2005; Meeuwig et al. 2010; Kanno et al. 2011a).

Microgeographic genetic differentiation

Microgeographic genetic differentiation occurs when population divergence exists within the dispersal limits of the species and suggests that other factors, such as barriers to gene flow or natural selection,

have acted to shape population structure within dispersal range (Richardson et al. 2014). For example, Tatarenkov et al. (2010) discovered that adjacent demes of a freshwater cyprinid, the swordtail (*Xiphophorus helleri*), displayed patterns of microgeographic differentiation because waterfalls interrupted gene flow. Additionally, although natural selection acts upon traits, selection could affect neutral genetics by selecting against certain genotypes that influence movement or dispersal. Richardson & Urban (2013), for example, found that predation risk among ponds significantly associated with neutral genetic differentiation for the spotted salamander (*Ambystoma maculatum*), demonstrating that selective forces could create patterns of microgeographic differentiation. I detected genetic differentiation at distances within the estimated dispersal range of 400 km. This microgeographic differentiation occurred within watersheds and across relatively fine spatial scales within the Kuparuk and Sagavanirktok watersheds compared to grayling high dispersal capabilities.

Dry zones as barriers

I found a strong association between fine scale genetic differentiation and extent of river drying after controlling for isolation by distance, suggesting that dry river zones might facilitate patterns of microgeographic differentiation for Arctic grayling, either by acting as barriers or through selective forces. Physical barriers often play a role in patterning fine-scale structure of freshwater fish populations (Whiteley et al. 2006; Kanno et al. 2011a; Junker et al. 2012; Junge et al. 2014), but the degree to which gene flow is disrupted depends largely on the dispersal ability of the species and the permeability of the barrier (Bergerot et al. 2015). Arctic grayling are capable of long distance dispersal, as evidenced by genetic similarities between the Itkillik and Ublutuoch fish, sampled 379 km apart. Yet, I found differentiation among fish distributed across dry zones at scales of less than x km. These patterns suggest that dry river zones might act as physical barriers to gene flow. Similar to findings by Kanno et al. (2011) for brook trout and Whiteley et al. (2006) for bull trout, dry zone barriers might reduce gene flow, increasing the effects of genetic drift. Although I found evidence supporting isolation of locations

separated by dry river zones, dry zones did not impede all fish movement, as indicated by mixed assignment probabilities at multiple locations.

Arctic tundra streams might behave more similarly to drought-prone desert aquatic systems, where stream distance and river intermittency best predicted genetic divergence among sites for desert fish (Fitzpatrick et al. 2014). In my study system, river drying often occurs when low precipitation and high evapotranspiration rates lead to low stream flow, resulting in aquatic habitat fragmentation (Betts & Kane 2015), which could temporarily restrict fish movement. These conditions most often occur during the Arctic summer and fall and not during the spring freshet when Arctic grayling leave overwintering areas to spawn. Thus, dry zones might not directly impede gene flow, but rather detainment of fish by river drying might alter demographic rates through increased mortality of adult and larval grayling that venture within or beyond drought prone river reaches.

Metapopulation structure and dynamics

In general, a metapopulation consists of local populations of a species existing within a variable regional environment, where local populations persists through dispersal among suitable habitat patches despite local population extinctions (Hanski & Simberloff 1997). High levels of fragmentation within a metapopulation might lead to local population isolation, decreased effective population size and loss of genetic diversity within populations, but some degree of fragmentation might increase overall genetic variability (Hanski 2011). Based on estimates of genetic clustering, migration rates and effective population sizes, I suggest that an inverse mainland-island metapopulation, as described by Altermatt & Ebert (2010), might best depict present Arctic grayling population structure. Hanski & Simberloff (1997) define the mainland-island metapopulation as a system of habitat patches (islands) located within dispersal distance from a very large habitat patch (mainland), where the mainland patch has a high persistence probability and provides a source of emigrants to island patches. Ross (2006) showed that mainland-like patches influenced persistence of the metapopulation in both static and dynamic landscape models due to its connectivity to island patches. Although I found multiple, small subpopulations

connected to a single large population, dispersal from the large population to island populations was only three percent, while dispersal from islands populations to the mainland was 17 percent. Altermatt & Ebert (2010) coined the term "inverse mainland-island" to describe similar metapopulation structure for *daphnia magna* in freshwater ephemeral pools along the Baltic Sea. They found that small populations in environmentally variable habitat patches produced proportionally more migrants than large, long-lived populations. They suggested that inverse mainland-island metapopulation dynamics might arise if species' traits affecting local survival negatively correlated with traits affecting migration. In Arctic tundra streams, drought-prone island patches likely produce conditions that negatively correlate with Arctic grayling survival (i.e. warm temperatures, deoxygenation), possibly prompting migration from island patches to the mainland patch.

Wilson & Rannala (2003) suggest that highly asymmetric migration results from their program, BAYESASS3, might indicate source-sink population dynamics, possibly reflecting habitat specific demographic (birth and death) and dispersal patterns among heterogeneous environments. At the population level within a metapopulation, local adaptation and environmental stochasticity can interplay, changing demographic rates within habitat patches so that sink populations transform into potential source populations and visa versa (Holt 2011). Although I found asymmetric dispersal among genetic clusters (i.e. Kuparuk and Toolik) and sampled locations (i.e. Campsite and Oksrukuyik Creek), assessment of habitat suitability was beyond the scope of this study. Understanding the asymmetry of migration rates within my study system requires further investigation, including spatial and temporal assessment of population movement patterns, vital rates, abundance and capacity to adapt to changing conditions.

Climate change and metapopulation dynamics

Arctic freshwater metapopulations might be particularly vulnerable to climate change conditions due to potential effects of warmer summers on hydrology and aquatic connectivity across the North Slope. Due to changes in precipitation and evapotranspiration rates, anticipated increases in frequency and duration of

river dry zones (Larry D. Hinzman et al. 2005b; Kane et al. 2004; Erica D Betts & Kane 2015) will likely alter the current Arctic grayling metapopulation structure. Analogous to birth and death rates for local population viability, the balance between colonization and extirpation rates of local populations determines metapopulation persistence. Changes in hydrology due to climate change will likely alter aquatic habitat connectivity, influencing local population dynamics and gene flow, thereby altering colonization and extirpation rates within the metapopuation. Although current Arctic grayling population structure suggests isolation of headwater populations with increased extirpation risk, these marginal local populations might provide the metapopulation with important sources of novel genetic variation, produced through isolation and local adaptation (Holt 2011). The existence of a large mainland population receiving migrants from potentially locally adapted island populations presents an interesting scenario, such that the mainland might act as a genetic reservoir for novel alleles necessary to recolonize extirpated habitat patches. Although I found very low migration rates from the mainland population to island populations, simulation models have shown that even a small number of migrants per year (3 to 5 adults) will allow individual populations to persist in stochastic environments where they would otherwise quickly go extinct (Stacey et al. 1997). The variability of the Arctic ecosystem, with rivers drying in summer, freezing in winter and changing course during flood events, continually provides stochastic environments that require organisms to either relocate or adapt to changing conditions. The answer to Arctic grayling persistence in the uncertain future of climate change depends on balances between gene flow, drift, local adaptation and population demographic rates, which all rely upon degree of aquatic habitat connectivity.

Conclusions

In this study, I examined the role of environmental factors structuring freshwater fish populations. I found a high degree of population structure with populations connected by complex patterns of gene flow. Dispersal from ancient refugia, past glacial activity and ephemeral river dry zones explained much of the genetic differentiation and asymmetric migration among local populations. In particular, evidence

suggests that river dry zones restrict fish movement, thereby increasing genetic divergence among local populations. Presence of a genetic mainland reservoir population, however, might foster resilience for the metapopulation provided low levels of migration to island populations or to extirpated habitat patches persist. This study underscores the importance of understanding genetic structure, species dispersal and habitat connectivity for metapopulation persistence and species management and conservation.

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Figures

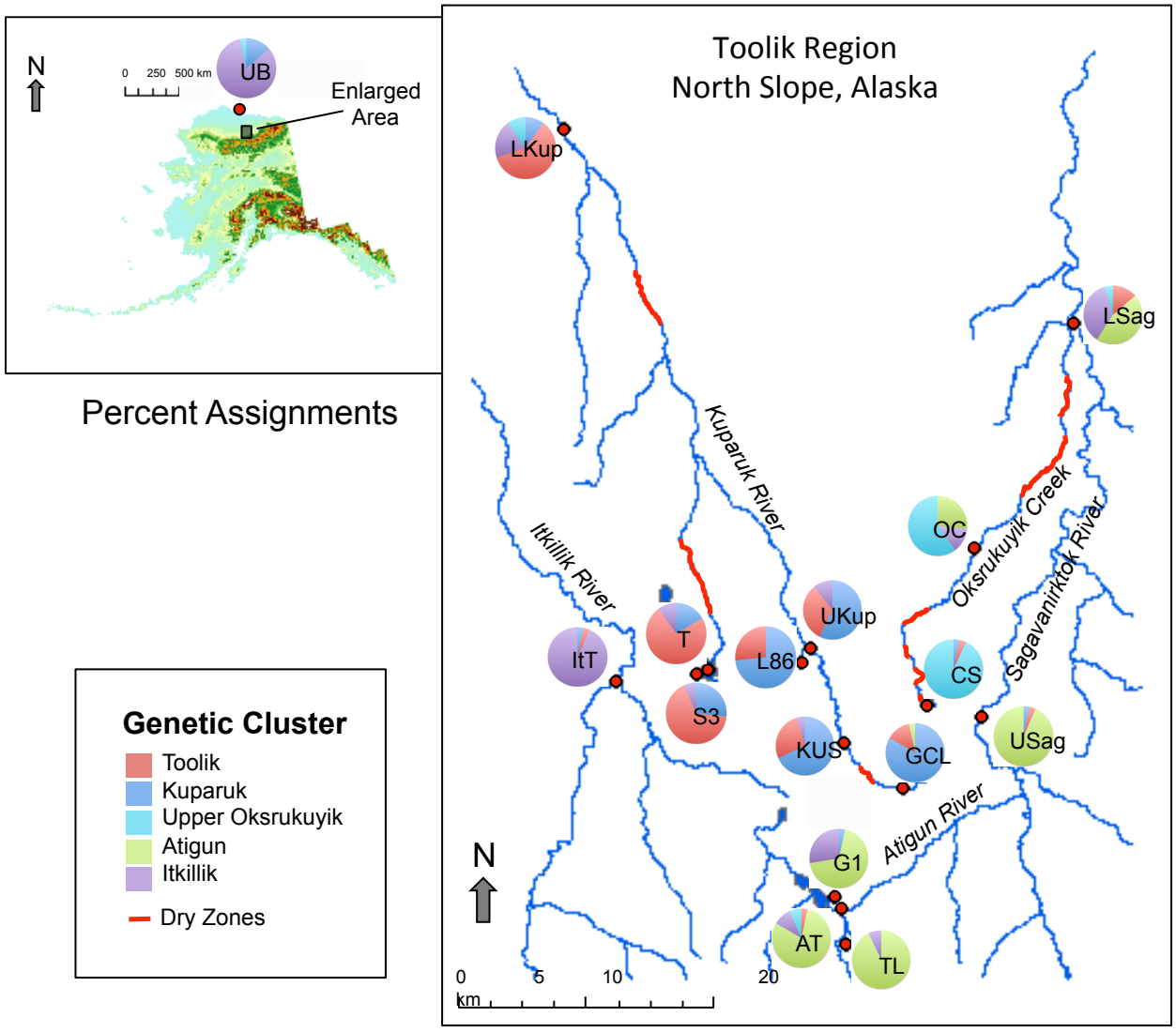

Figure 1. Study area and population structure. Red dots indicate 16 Arctic grayling sampling locations. Pie charts depict population assignments to five genetic clusters (AT = Atigun River; G1 = Galbraith Lake 1; TL = Tea Lake; USag = Upper Sagavanirktok River, LSag = Lower Sagavanirktok River; OC = Oksrukuyik Creek; CS = Campsite Lake; UB = Ublutuoch River; IT = Itkillik River; GCL = Green Cabin Lake; KUS = Kuparuk Upper Spring; UKup = Upper Kuparuk River; L86 = Lake 86; LKup = Lower Kuparuk River; S3 = Lake S3; T = Toolik Lake).

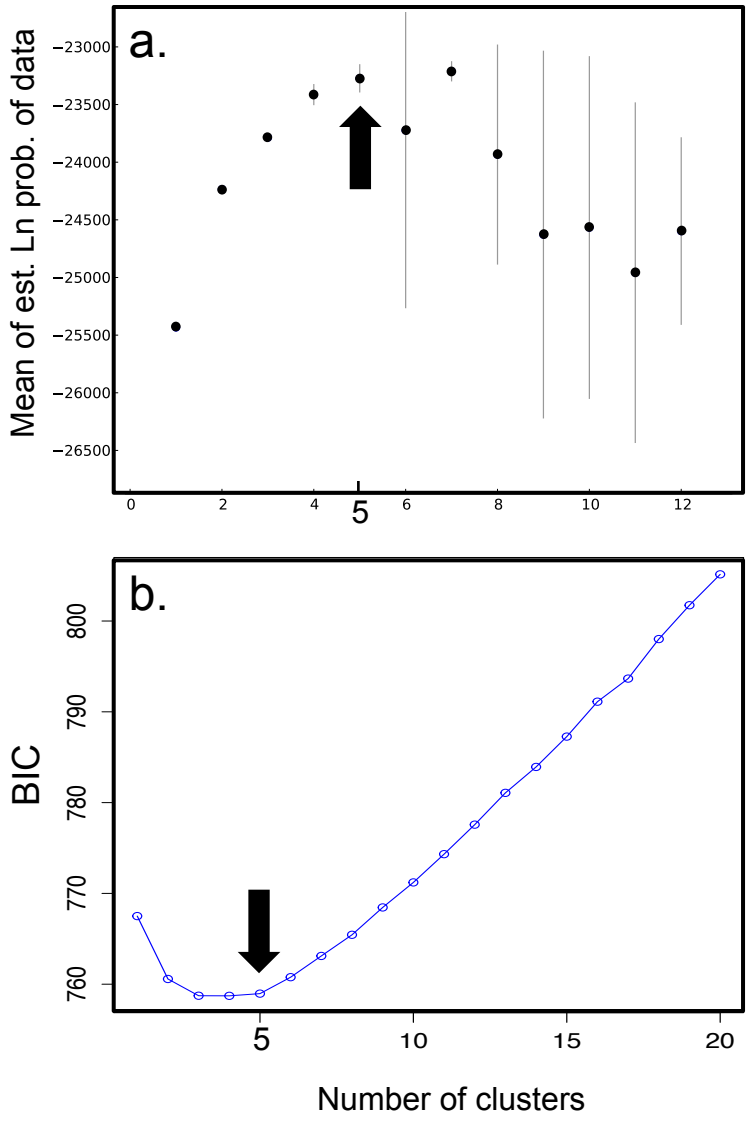

Figure 2. Clustering results: a. mean log probability of the data from Structure and b. Bayesian information criterion (BIC) from DAPC.

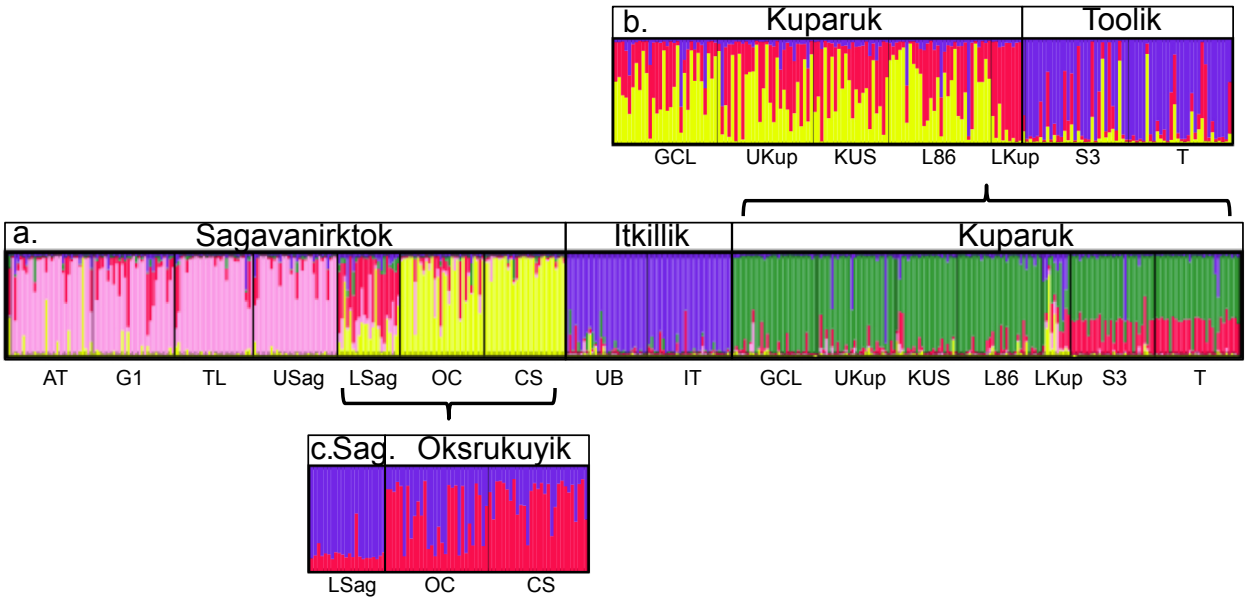

Figure 3. STRUCTURE plots for (a) K = 5 genetic clusters: 16 locations within three main watersheds (Sagavanirktok, Itkillik and Kuparuk); (b) K = 3 genetic clusters: 7 locations within two sub-watersheds (Kuparuk and Toolik); and (c) K = 2 genetic clusters: 3 locations including the Lower Sagavanirktok River (LSag) and Oksrukuyik sub-watershed. Each vertical bar in the STRUCTURE graph represents an individual and colors indicate percent assignment of each individual to each genetic clusters (colors). Black vertical lines separate sample locations (AT = Atigun River; G1 = Galbraith Lake 1; TL = Tea Lake; USag = Upper Sagavanirktok River, LSag = Lower Sagavanirktok River; OC = Oksrukuyik Creek; CS = Campsite Lake; UB = Ublutuoch River; IT = Itkillik River; GCL = Green Cabin Lake; UKup = Upper Kuparuk River; KUS = Kuparuk Upper Spring; L86 = Lake 86; LKup = Lower Kuparuk River; S3 = Lake S3; T = Toolik Lake).

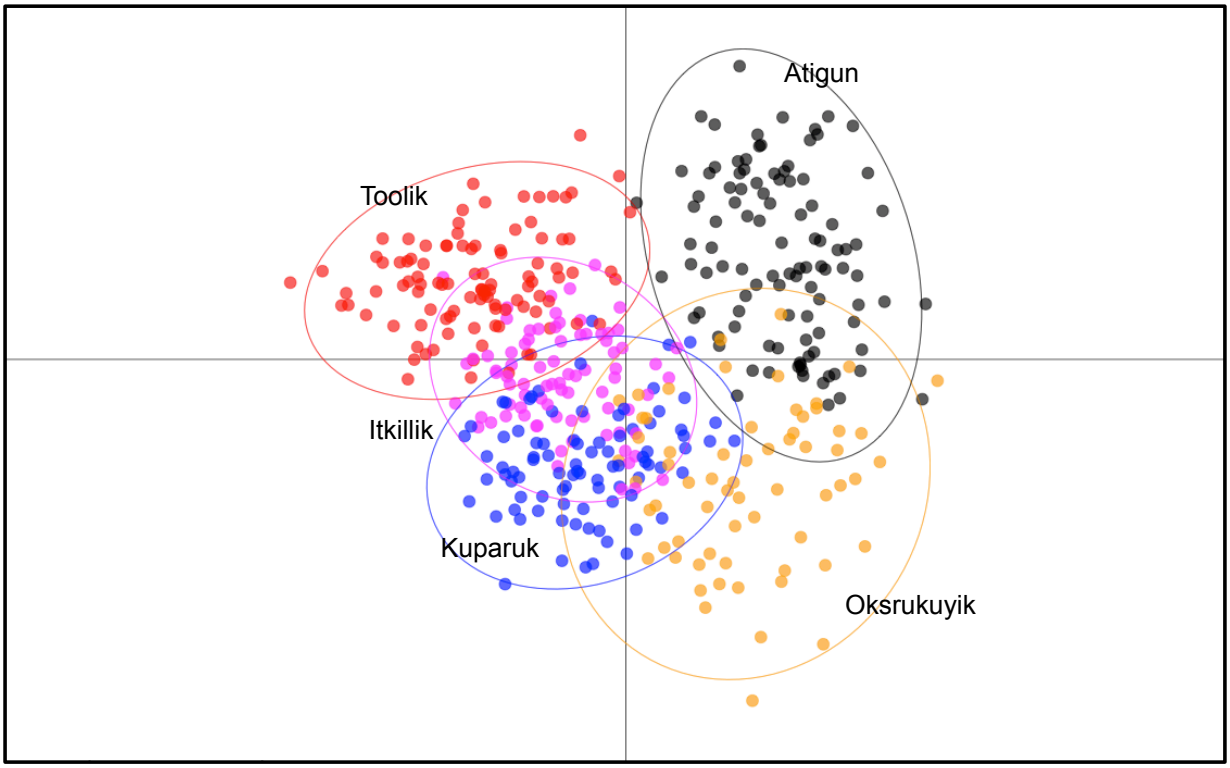

Figure 4. Discriminant analysis of principal components, the first two axes plotted with five genetic clusters (main graph) and three genetic clusters (inset). Labels indicate geographic location to which each cluster was most highly associated.

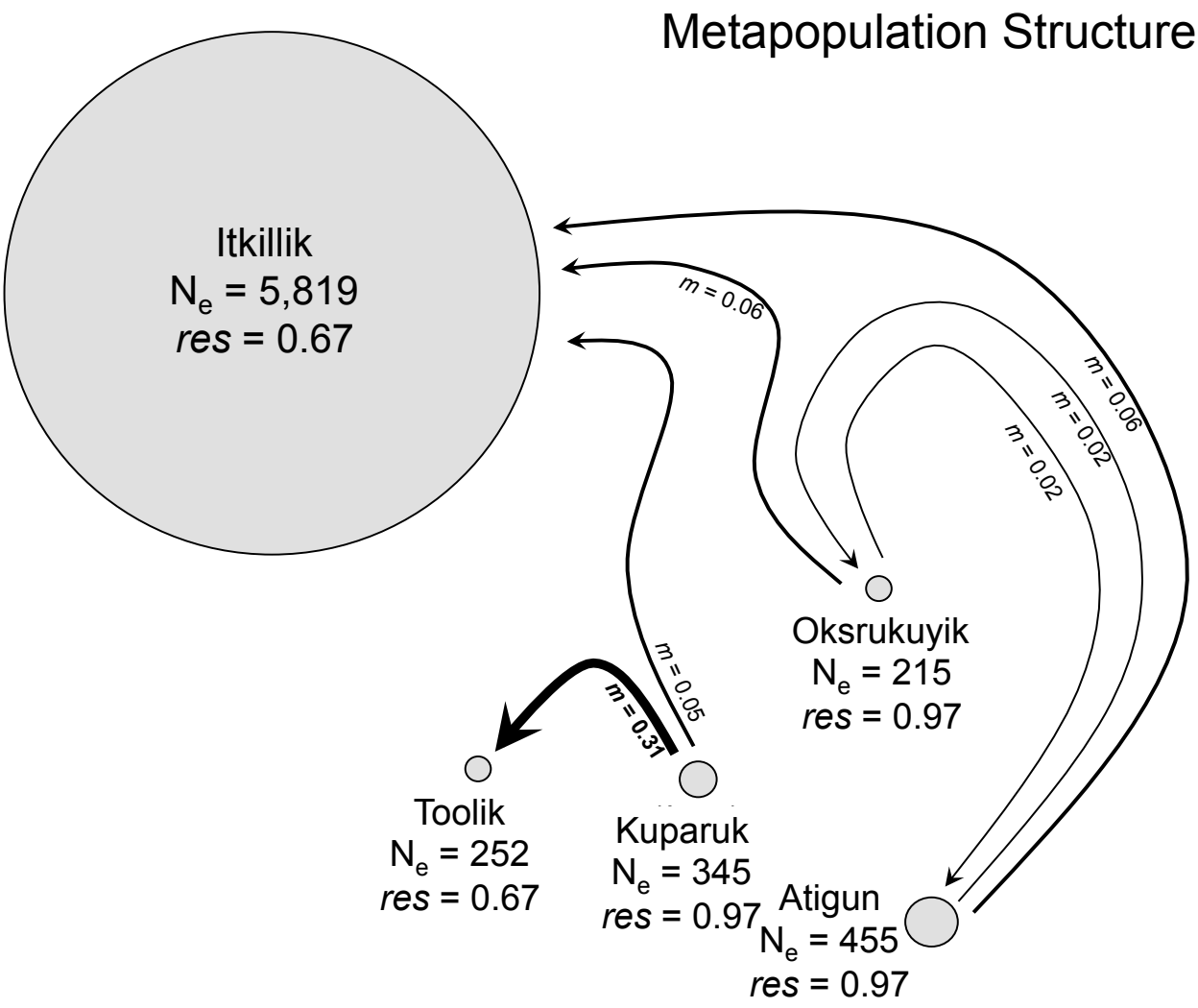

Figure 5. BAYESASS estimates of migration rates (*m*) among the five genetic clusters. Only migration rates over 1% are shown here (see Table 4 for pairwise migration rates between all genetic clusters). Line thickness represents migration rate, and circle size indicates approximate population size based on *NeEstimator* estimates of effective population size (N_e) (see Table 3 for effective population size confidence intervals for all genetic clusters). Residency (res) refers to the proportion of non-migrants and reflects the rate at which genes originating from within the local genetic cluster. The five genetic clusters are identical to those used in DAPC (Fig. 4).

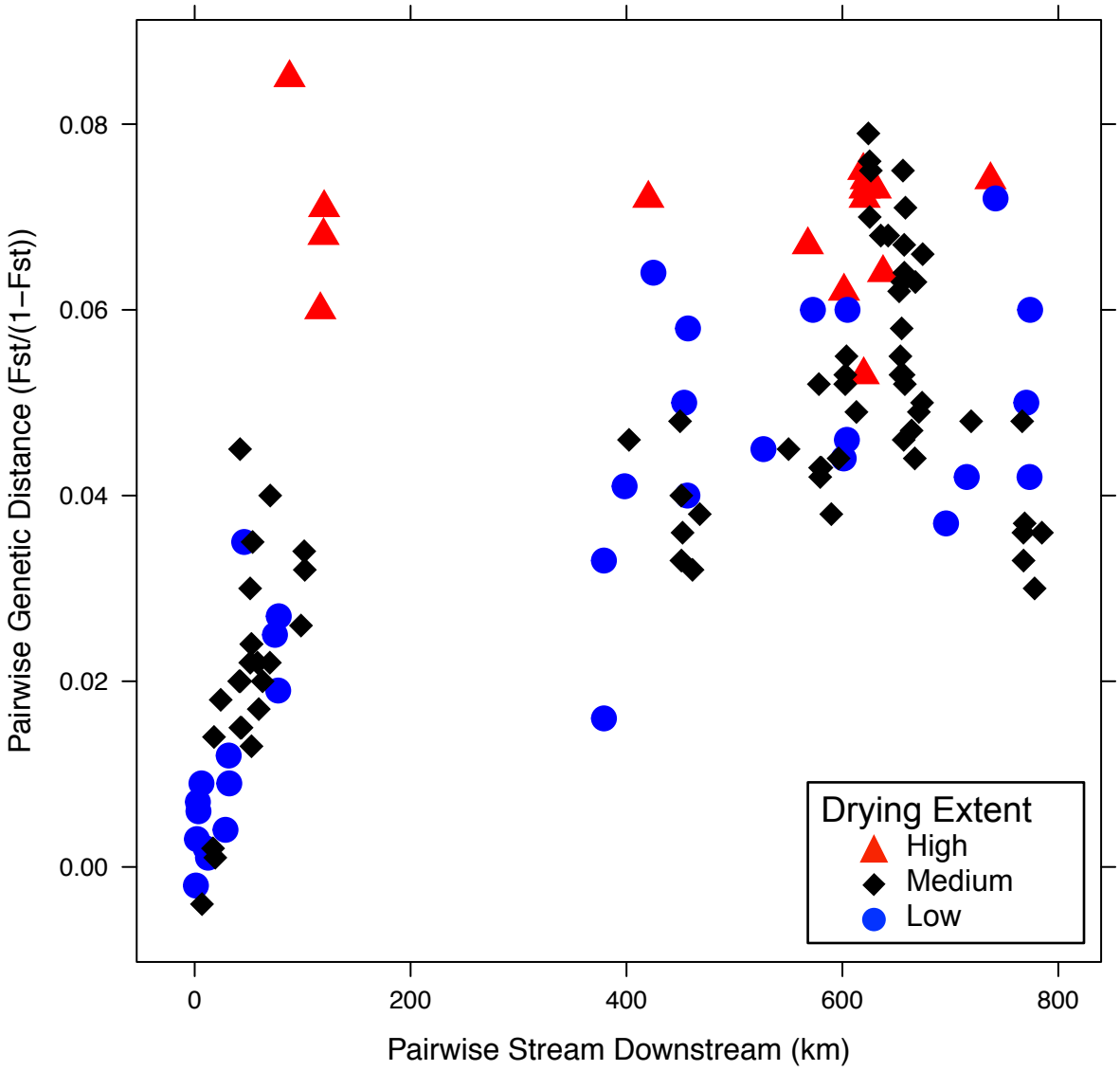

Figure 6. Genetic distance versus pairwise geographic stream distance, with extent of river drying. Shapes and colors represent different extent of river drying between pairs of locations: Blue circles = 0 km (low), black diamonds = 1 – 15 km (medium) and red triangles > 15 km (high). The inset graph is taken from Koizumi et al. (2006) and indicates different patterns expected under various drift versus gene flow scenarios.

Tables

Table 1. Geographic location and genetic diversity indices for 16 sampling sites. Column abbreviations for genetic diversity: n, number of individuals per sample; A, number of alleles; A_{eff}, effective number of alleles; A_r, rarified allelic richness; PrA_r, rarified private allele richness; H_O, observed heterozygostiy; H_E, expected heterozygosity.

Location	ID	Latitude	Longitude	Elevaiton (m)	n	A	A_{eff}	A_r	PrA_r	H_o	H_e
Atigun	AT	68.451597°	-149.373811°	794	30	15	8	6.5	0.3	0.877	0.880
Lake G1	G1	68.460939°	-149.385639°	800	28	16	9	6.8	0.3	0.886	0.900
Tea Lake	TL	68.425199°	-149.369217°	817	28	13	7	6.2	0.2	0.864	0.866
Upper Sagavanirktok	USag	68.583650°	-149.069289°	593	30	16	7	6.3	0.4	0.823	0.865
Lower Sagavanirktok	LSag	68.865861°	-148.838862°	403	22	15	10	7.2	0.4	0.927	0.912
Oksrukuyik Creek	OC	68.707349°	-149.064982°	729	30	14	8	6.5	0.3	0.887	0.888
Campsite Lake	CS	68.597042°	-149.181145°	862	29	13	6	5.8	0.2	0.797	0.836
Ublutuoch	UB	70.319766°	-151.354145°	0	29	19	13	7.6	1.0	0.917	0.934
Itkillik Tributary	IT	68.624738°	-149.792362°	617	30	20	13	7.6	1.3	0.920	0.933
Green Cabin Lake	GCL	68.536452°	-149.236909°	878	30	13	8	6.4	0.2	0.847	0.884
Kuparuk Upper Spring	KUS	68.572448°	-149.347503°	815	24	14	9	6.8	0.2	0.891	0.901
Upper Kuparuk River	UKup	68.645579°	-149.407595°	736	28	15	9	6.8	0.2	0.896	0.897
Lake 86	L86	68.632599°	-149.419062°	797	29	15	9	6.6	0.2	0.863	0.889
Lower Kuparuk River	LKup	69.021332°	-149.798617°	340	9	11	7	6.7	0.4	0.890	0.882
Lake S3	S3	68.628592°	-149.625314°	720	29	15	8	6.6	0.2	0.853	0.889
Toolik Lake	T	68.632190°	-149.605977°	717	30	14	8	6.3	0.2	0.880	0.871

Table 2. Pairwise F_{ST} (lower triangle) and chi square significance (p-value) for pairwise F_{ST} (upper triangle).

	AT	G1	TL	USag	LSag	OC	CS	UB	IT	GCL	KUS	UKup	L86	LKup	S3	T
AT	-	0.074	0.040	0.064	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.
G1	0.007	-	0.007	0.000	0.000	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.
TL	0.006	0.009	-	0.000	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.
USag	0.004	0.012	0.009	-	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.
LSag	0.024	0.019	0.026	0.034	-	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.
OC	0.025	0.033	0.031	0.038	0.018	-	0.000	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.
CS	0.057	0.064	0.066	0.078	0.043	0.014	-	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.
UB	0.048	0.038	0.055	0.060	0.032	0.044	0.067	-	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.
IT	0.048	0.040	0.057	0.067	0.036	0.046	0.069	0.016	-	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.	highly sig.
GCL	0.047	0.048	0.062	0.064	0.042	0.050	0.060	0.037	0.035	-	0.528	0.121	0.074	highly sig.	highly sig.	highly sig.
KUS	0.045	0.042	0.059	0.064	0.037	0.047	0.068	0.031	0.029	0.004	-	0.132	0.134	highly sig.	highly sig.	highly sig.
UKup	0.050	0.044	0.060	0.065	0.040	0.050	0.067	0.032	0.032	0.002	0.002	-	0.482	0.000	0.000	highly sig.
L86	0.055	0.049	0.066	0.070	0.041	0.052	0.069	0.035	0.036	0.001	0.001	0.003	-	highly sig.	highly sig.	highly sig.
LKup	0.042	0.044	0.057	0.057	0.043	0.043	0.063	0.039	0.040	0.022	0.020	0.023	0.034	-	highly sig.	highly sig.
S3	0.052	0.050	0.063	0.071	0.041	0.049	0.068	0.038	0.035	0.017	0.013	0.015	0.015	0.023	-	0.786
T	0.058	0.059	0.070	0.073	0.049	0.058	0.070	0.046	0.046	0.022	0.022	0.020	0.020	0.029	0.002	-

Table 3. Estimates of effective population size five genetic clusters determined using DAPC. P_{Crit} (1/2*S*, where *S* is the sample size) determines the level for the lowest allele frequency used. Confidence intervals are indicated in parentheses.

			Lowest Allele Frequency Used			
	S	P_{Crit}	**0.05**	**0.02**	**0.01**	**0+**
Itkillik	89	0.006	Infinite	887.8	895	**5818.8**
			(684, Infinite)	(482.3, 4571.9)	(526.2, 2768.8)	**(1043.3, Infinite)**
Toolik	86	0.006	260	403	428.8	**251.7**
			(142.8, 950.1)	(257.3, 865.6)	(295.8, 752.5)	**(189, 367.2)**
Oksrukuyik	51	0.010	233.2	234.1	**214.9**	137.6
			(94.9, Infinite)	(130.1, 878.8)	**(133.5, 497.3)**	(96.3, 228.2)
Atigun	115	0.004	Infinite	1460.7	1918	**455.2**
			(3350.4, Infinite)	(567, Infinite)	(754.5, Infinite)	**(285.5, 1020.5)**
Kuparuk	96	0.005	1361.2	862.8	725	**344.7**
			(326.1, Infinite)	(461.7, 5020.2)	(446.1, 1812.8)	**(229.7, 649.3)**

Table 4. Estimates of effective population size for each of 16 sampling locations. P_{Crit} (1/2*S*, where *S* is the sample size) determines the level for the lowest allele frequency used. Confidence intervals are indicated in parentheses.

			Lowest Allele Frequency Used			
	S	P_{Crit}	**0.05**	**0.02**	**0.01**	**0+**
AT	30	0.02	537.9	**415.3**	637.5	637.5
			(111.2, Infinite)	***(123.6, Infinite)***	*(172, Infinite)*	*(172, Infinite)*
G1	29	0.02	Infinite	**Infinite**	1074.6	1074.6
			(247.3, Infinite)	***(415.2, Infinite)***	*(183.6, Infinite)*	*(183.6, Infinite)*
TL	28	0.02	Infinite	**Infinite**	175.9	175.9
			(269.8, Infinite)	***(243.6, Infinite)***	*(97.4, 719.8)*	*(97.4, 719.8)*
US	30	0.02	Infinite	**Infinite**	297.4	297.4
			(155.2, Infinite)	***(213.8, Infinite)***	*(115.1, Infinite)*	*(115.1, Infinite)*
LS	22	0.02	947.4	**Infinite**	Infinite	Infinite
			(95.1, Infinite)	***(286.4, Infinite)***	*(286.4, Infinite)*	*(286.4, Infinite)*
OC	30	0.02	53.9	**53.4**	73.5	73.5
			(36.8, 91.9)	***(42.6, 70.2)***	*(53.1, 114.3)*	*(53.1, 114.3)*
CS	29	0.02	3134.6	**508.4**	295.6	295.6
			(78.2, Infinite)	***(99.6, Infinite)***	*(106.8, Infinite)*	*(106.8, Infinite)*
UB	29	0.02	Infinite	**Infinite**	Infinite	Infinite
			(458.5, Infinite)	***(3232.1, Infinite)***	*(Infinite, Infinite)*	*(Infinite, Infinite)*
IT	30	0.02	157.8	**319.8**	Infinite	Infinite
			(79.6, 1541.7)	***(149.9, Infinite)***	*(486.7, Infinite)*	*(486.7, Infinite)*
GCL	30	0.02	298.5	**270.1**	645.2	645.2
			(111.6, Infinite)	***(128.2, Infinite)***	*(193.6, Infinite)*	*(193.6, Infinite)*
KUS	22	0.02	Infinite	**Infinite**	Infinite	Infinite
			(344, Infinite)	***(37545.1, Infinite)***	*(37545.1, Infinite)*	*(37545.1, Infinite)*
UKup	28	0.02	413.2	**1781.4**	Infinite	Infinite
			(97.7, Infinite)	***(147.8, Infinite)***	*(246.5, Infinite)*	*(246.5, Infinite)*
L86	30	0.02	271.8	**213.3**	272.3	272.3
			(111.6, Infinite)	***(106.2, Infinite)***	*(129.9, Infinite)*	*(129.9, Infinite)*
LKup	10	0.05	**Infinite**	Infinite	Infinite	Infinite
			(Infinite, Infinite)	*(Infinite, Infinite)*	*(Infinite, Infinite)*	*(Infinite, Infinite)*
S3	30	0.02	510.6	**219.7**	302.8	302.8
			(119.4, Infinite)	***(101.4, Infinite)***	*(123.6, Infinite)*	*(123.6, Infinite)*
T	30	0.02	75.5	**123.1**	135.4	135.4
			(45.9, 177.4)	***(76.3, 286.8)***	*(85.8, 297.1)*	*(85.8, 297.1)*

Table 5. Migration rates from BayesAss3 analysis of five genetic clusters determined using DAPC. The fraction of individuals from population i (rows) that are migrants derived from population j (columns) per generation. Values > 0.01 shown.

	Itkillik		**Toolik**		**Oksrukuyik**		**Atigun**		**Kuparuk**	
Itkillik	**0.829**	0.020	0.005	0.005	0.055	0.016	0.059	0.016	0.052	0.015
Toolik	0.004	0.004	**0.670**	0.004	0.009	0.006	0.010	0.006	0.307	0.010
Oksrukuyik	0.007	0.006	0.006	0.006	**0.965**	0.016	0.015	0.012	0.007	0.007
Atigun	0.003	0.003	0.003	0.003	0.021	0.011	**0.969**	0.012	0.004	0.003
Kuparuk	0.013	0.007	0.004	0.004	0.006	0.005	0.012	0.007	**0.966**	0.011

Table 6. Migration rates (and standard deviation) from BayesAss3: The fraction of individuals in population *i* (rows) that are migrants derived from population *j* (columns) per generation. Values < 0.01 not shown.

	AT	G1	TL	USag	LSag	OC	CS	UB	IT	GCL	KUS	UKup	L86	LKup	S3	T
AT	0.86 *0.03*						0.04 *0.02*									
G1	0.21 *0.02*	0.67 *0.01*														
TL	0.22 *0.02*		0.67 *0.01*													
USag	0.22 *0.02*			0.67 *0.01*												
LSag	0.08 *0.03*				0.68 *0.01*		0.11 *0.04*									
OC						0.67 *0.01*	0.22 *0.02*									
CS	0.04 *0.03*						0.85 *0.03*									
UB								0.79 *0.04*	0.04 *0.02*	0.04 *0.02*						
IT								0.08 *0.03*	0.77 *0.03*	0.04 *0.02*						
GCL										0.88 *0.03*						
KUS										0.20 *0.03*	0.68 *0.01*					
UKup										0.22 *0.03*		0.67 *0.01*				
L86										0.22 *0.02*			0.67 *0.01*			
LKup							0.02 *0.02*			0.12 *0.03*				0.68 *0.01*		0.02 *0.02*
S3										0.06 *0.02*					0.67 *0.01*	0.17 *0.03*
T										0.07 *0.03*						0.82 *0.03*

Supplemental Tables

Table S1. Pairwise river distance (km) (upper triangle) and pairwise dry zone extent (km) (lower triangle) among 16 sample locations.

	AT	G1	TL	USag	LSag	OC	CS	UB	IT	GCL	KUS	UKup	L86	LKup	S2	T
AT	-	3	4	29	74	98	116	453	770	671	664	654	655	601	654	653
G1	0	-	6	31	77	101	119	456	773	674	667	657	658	604	657	656
TL	0	0	-	32	78	102	120	457	774	674	668	657	658	605	657	656
USag	0	0	0	-	46	70	88	425	742	642	636	625	626	573	625	624
LSag	0	0	0	0	-	24	42	379	696	597	590	579	581	527	579	578
OC	19.2	19.2	19.2	19.2	19.2	-	18	402	719	620	613	603	604	550	603	601
CS	20.6	20.6	20.6	20.6	20.6	1.4	-	420	737	638	631	621	622	568	621	619
UB	0	0	0	0	0	19.2	20.6	-	379	468	461	451	452	398	451	450
IT	0	0	0	0	0	19.2	20.6	0	-	785	778	768	769	715	768	767
GCL	3.3	3.3	3.3	3.3	3.3	22.4	23.8	3.3	3.3	-	7	17	19	70	59	58
KUS	2.7	2.7	2.7	2.7	2.7	21.9	23.3	2.7	2.7	0.5	-	10	12	63	53	51
UKup	2.7	2.7	2.7	2.7	2.7	21.9	23.3	2.7	2.7	0.5	0	-	2	53	42	41
L86	2.7	2.7	2.7	2.7	2.7	21.9	23.3	2.7	2.7	0.5	0	0	-	54	43	42
LKup	0	0	0	0	0	19.2	20.6	0	0	3.3	2.7	2.7	2.7	-	53	51
S2	7.2	7.2	7.2	7.2	7.2	26.4	27.8	7.2	7.2	5	4.5	4.5	4.5	7.2	-	1
T	7.2	7.2	7.2	7.2	7.2	26.4	27.8	7.2	7.2	5	4.5	4.5	4.5	7.2	0	-

Table S2. Pairwise elevation difference (m) among 16 sample locations.

	AT	G1	TL	USag	LSag	OC	CS	UB	IT	GCL	KUS	UKup	L86	LKup	S3	T
AT	-	-6	-23	201	391	65	-68	794	177	-84	-21	58	-3	454	74	77
G1	6	-	-17	207	397	71	-62	800	183	-78	-15	64	3	460	80	83
TL	23	17	-	224	414	88	-45	817	200	-61	2	81	20	477	97	100
USag	-201	-207	-224	-	190	-136	-269	593	-24	-285	-222	-143	-204	253	-127	-124
LSag	-391	-397	-414	-190	-	-326	-459	403	-214	-475	-412	-333	-394	63	-317	-314
OC	-65	-71	-88	136	326	-	-133	729	112	-149	-86	-7	-68	389	9	12
CS	68	62	45	269	459	133	-	862	245	-16	47	126	65	522	142	145
UB	-794	-800	-817	-593	-403	-729	-862	-	-617	-878	-815	-736	-797	-340	-720	-717
IT	-177	-183	-200	24	214	-112	-245	617	-	-261	-198	-119	-180	277	-103	-100
GCL	84	78	61	285	475	149	16	878	261	-	63	142	81	538	158	161
KUS	21	15	-2	222	412	86	-47	815	198	-63	-	79	18	475	95	98
UKup	-58	-64	-81	143	333	7	-126	736	119	-142	-79	-	-61	396	16	19
L86	3	-3	-20	204	394	68	-65	797	180	-81	-18	61	-	457	77	80
LKup	-454	-460	-477	-253	-63	-389	-522	340	-277	-538	-475	-396	-457	-	-380	-377
S3	-74	-80	-97	127	317	-9	-142	720	103	-158	-95	-16	-77	380	-	3
T	-77	-83	-100	124	314	-12	-145	717	100	-161	-98	-19	-80	377	-3	-

Table S3. Pairwise watershed movement: among same watershed = 0, among different watersheds = 1).

	AT	G1	TL	USag	LSag	OC	CS	UB	IT	GCL	UKup	KUS	L86	LKup	S3	T
AT	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1
G1	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1
TL	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1
USag	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1
LSag	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1
OC	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1
CS	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1
UB	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1
IT	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1
GCL	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0
UKup	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0
KUS	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0
L86	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0
LKup	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0
S3	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0
T	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0

Chapter 2:

Microgeographic neutral genetic differentiation predicts spawning displacement in a freshwater migratory fish

Abstract

Microgeographic genetic differentiation occurs when strong selection overwhelms gene flow in otherwise apparently well-connected systems. Understanding traits under selection in populations expressing microgeographic differentiation possess challenges, particularly when traits first appear in adulthood. In this study, I examined microgeographic differentiation in relation to adult migration propensity in the highly migratory species, Arctic grayling (*Thymallus arcticus*). I found significant within watershed differentiation for larval Arctic grayling in two different river systems: the Kuparuk and Oksrukuyik watersheds. PIT-tagged adults expressed variation in migration distance, which corresponded to fine-scale neutral genetic differentiation in the Kuparuk watershed. Both watersheds consisted of distinct headwater and downstream populations that exhibited differences in distance migrated and directionality of movement. Movement patterns of spawning adults might have evolved through selection within a variably adaptive aquatic landscape, which holds implications for Arctic grayling local population persistence with a rapidly changing Arctic climate.

Introduction

Microgeographic genetic differentiation occurs when population divergence exists within the dispersal limits of the species and suggests that evolutionary forces, such as natural selection,

have acted to shape population structure within dispersal range (Richardson & Urban 2013). In a well connected landscape where gene flow is strong, the effects of gene flow are expected to decrease the effects of drift and selection, thereby reducing genetic differentiation among locations (Kawecki & Ebert 2004). Nevertheless, examples of microgeographic differentiation span the plant and animal kingdom (Linhardt & Grant 1996; Bilton et al. 2002; Fraser et al. 2011), signifying that strong evolutionary forces often lead to genetic divergence of subpopulations despite apparent gene flow. In salamander populations, for instance, Richardson & Urban (2013) found that even within apparently well-connected landscapes gene flow was altered by cryptic selective predation levels among ponds, leading to fine-scale neutral genetic differentiation of populations. Yet with mounting indication of the occurrence of microgeographic differentiation, we still do not fully understand the importance of fine-scale population structure and the significance of trait selection in species evolution and persistence.

Perhaps as a consequence of limited dispersal pathways, freshwater systems have revealed many incidences of fine-scale genetic differentiation. Differentiation in river systems is often associated with breaks in aquatic connectivity. For example, Tatarenkov et al. (2010) discovered microgeographic differentiation in green swordtail (*Xiphophorus helleri*) populations, which they attributed to waterfall barriers and small population size. Similarly, Kanno et al. (2011) found fine-scale hierarchical genetic structure in brook trout (*Salvelinus fontinalis*) attributable to seasonal waterfall barriers, but they found differentiation due to unobstructed tributary confluences at distances of only a few km, as well. Likewise, Koskinen et al. (2001) found genetic structure on a small geographic scale for the highly mobile European grayling (*Thymallus thymallus*) at different spawning sites around Lake Saimaa, Finland, despite absence of physical barriers. And similarly, Reilly et al. (2014) discovered significant sub-basin genetic differentiation for Arctic grayling (*Thymallus arcticus*) notwithstanding the species high mobility and tendency for movement among spawning tributaries (Blackman 2002). Although research

regarding fine-scale genetic structure is essential to understanding generalized population structure in these dendritic systems, predicting the outcomes of environmental change and species management actions necessitates investigation of evolutionary drivers and species traits that are likely to come under selection.

In Arctic tundra streams, intermittently dry river zones (Betts & Kane 2015) provide a landscape comprised of drought-prone and drought-resistant segments, which might impose varying selection for or against certain phenotypes. Arctic grayling are known to successfully navigate these intermittently dry river zones during spring spawning migrations, when average annual river discharge peaks, and during periodic flood events (Deegan et al. unpublished). Variability in habitability of these river segments might provide a means for natural selection to act upon heritable traits. If selection alters gene flow, such as through selection against certain migrants or evolution of habitat preference, neutral population differentiation on a microgeographic scale might ensue. Yet, for long-lived species, like Arctic grayling, assessing heritability of adult trait variation on migrant phenotypes through common garden experiments remains impractical with time frames beyond the scope of most research grants. Thus, my study aims to associate an adult trait, movement distance, with microgeographic differentiation using neutral genetics in combination with PIT-tag technology.

This study examines microgeographic differentiation of Arctic grayling in tundra streams, using larval fish as a substitute for spawning stocks, and investigates an adult trait, spawning migration distance with regard to fine-scale neutral genetic differentiation. My null hypothesis states that adult Arctic grayling migrate from overwintering locations to spawning sites at random, with no specificity for certain locations or distances migrated. Alternatively, we hypothesize that if adult movement patterns suggest site specificity, then larval grayling genetics at spawning sites will most closely match adult grayling genetics with fidelity to those sites. Using passive integrated transponder (PIT) tagged adults, larvae as indicators of spawning

locations and neutral genetic analyses of larval and PIT-tagged adult individuals, we investigated Arctic grayling migration propensity as a phenotypic trait potentially under selection by a variable selective aquatic landscape. We predicted that (1) adult Arctic grayling express variation in migration distance from overwintering locations (2) larval grayling express microgeographic genetic differentiation as a result of spawning stock site fidelity and (3) adult migration distances and genetic signatures correlate positively with site-specific microgeographic genetic differentiation.

Materials and Methods

Natural History and Study Area

This study was conducted in two headwater streams located on the North Slope of the Brooks Mountain Range, Alaska: The Kupruk River and Oksrukuyik Creek (Figure 1). The Kuparuk River and Oksrukuyik Creek are clear water Arctic tundra streams consisting of alternating pool, run and riffle habitat. Both streams flow during the Arctic growing season from May to late September and freeze solid from mid-September to early May, except for a few spring locations where small pockets of water remain unfrozen year-round. Because deep lakes and springs provide the only overwintering habitat for stream-dwelling organisms, fish inhabiting streams during the open-water season must rely on an interconnected aquatic landscape to access suitable spawning, feeding and overwintering locations. Both the Kuparuk River and Oksrukuyik Creek are susceptible to drought, with large stretches of river drying occurring in years when evapotranspiration exceeds precipitation (Kane et al. 2004). When dry river zones occur, these areas are impassible and uninhabitable by fish occupying tundra streams during the summer growing season (Erica D Betts & Kane 2015).

The highly migratory salmonid, Arctic grayling, is the only species found within my study area, with the exception of the lowest reaches of Oksrukuyik Creek, where round whitefish

(*Prosopium cylindraceum)* and Arctic char (*Salvelinus alpinus)* are occasionally spotted. In both rivers, adult Arctic grayling tend to occupy deeper, fast-moving water in pools and runs, whereas age-0 grayling occupy shallower, low-current side and backwater areas of the rivers. Adult grayling make an annual spawning migration in the spring after ice-out occurs. Due to difficulty tracking fish during the spring freshet, Arctic grayling spawning locations for the Kuparuk River and Oksrukuyik Creek remain unknown. Newly emerged young-of-the-year Arctic grayling, however, provide an indication of spawning activity in these streams. Due to variation in habitability of reaches within each river, river drying might exert strong selection on Arctic grayling by increasing mortality of young, thereby decreasing fitness of individuals that spawn in these areas.

Fish Sampling

Adult and larval Arctic grayling were sampled from July 2010 to 2013 at fourteen sites across two watersheds that contained neutrally differentiated genetics: the Kuparuk River and Oksrukuyik Creek (Golden et al. in prep.). In order to assess spawning stock genetic structure within each watershed, I sampled post-emergent young-of-the-year Arctic grayling along each stream, including three locations within the Kuparuk River (Kup2, Kup6 and Kup8) and three locations within Oksrukuyik Creek (Oks0, Oks2 and Oks3) (Figure 1). Individual young-of-the-year Arctic grayling were collected for DNA analysis using dipnets at distances sufficient to ensure separation of families (at least > 10 m). Because actual spawning locations for adult Arctic grayling remain unknown, I assume post-emergent larval Arctic grayling will genetically represent spawning stocks within each river if they occur.

I captured adult Arctic grayling during the summer growing season using fyke nets and by angling. Adult Arctic grayling were anaesthetized in a eugenol solution (50 mg/L Aqui-SE®), weighed, measured, PIT-tagged and fin clipped for DNA analysis. I tagged fish using half-

duplex, 23-mm PIT-tags by making a small incision on the ventral side of the fish below the pelvic girdle and inserting the tag into the body cavity using a syringe. Once fully recovered, tagged fish were released back to the river near their sampling location. DNA samples from each fish were preserved in 95% ethanol, labeled with the fish's unique PIT-tag identification information and stored at -20° C until extractions were conducted.

PIT-tag antenna arrays

As part of collaborative research on fish movement patterns in the Kuparuk River, I established and maintained a series of stationary PIT-tag antenna arrays from the headwaters of the Kuparuk River to over 40 km downstream (Figure 1). Each PIT-tag antenna station consisted of an antenna, a tuner box, a marker tag, an Oregon RFID reader and a power station. The antenna consisted of a loop of antenna wire placed in cross-section within the stream channel that connected to a tuner box, which tuned the antenna to half-duplex PIT-tag frequency. The antenna and reader box were powered by three six-volt, deep-cycle, lithium batteries and recharged using solar panels. The marker tag was used to indicate when PIT-tag antennas ceased functioning due to environmental condition or power shortages. Although antenna arrays were installed up to 80 km downstream (Kup7 and Kup8 sample locations), these arrays were exceptionally difficult to maintain. Due to their inconsistent functionality, I removed them from the analysis, but instead included fish caught by angling at these locations as tag detections in the dataset. PIT-tag antenna arrays were deployed during the ice-free season (late May to mid-September) from 2010 to 2015.

Genotyping

DNA was extracted from adult fin and larval caudal tissue using DNeasy blood and tissue kits (Qiagen, CA). Multiplex PCR reactions were optimized for allelic range for eight highly variable nuclear microsatellite markers specific to Arctic grayling (Diggs & Ardren 2008) in a manner

similar to Steed (Steed 2007) (Table S1, supporting information). PCR products were analyzed on an ABI DNA sequencer and allele sizes were scored along with positive and negative controls using the program GeneMarker (SoftGenetics, LLC, State College, PA). All genotypes were hand-checked for accuracy. Amplifications that were too weak to resolve peaks or had excess stutter were re-amplified and rerun for better resolution. Any remaining unresolved alleles were treated as missing data.

Basic population genetic statistics

To assess microsatellite markers and provide descriptive statistics, I used the program PopGenReport (Adamack & Gruber 2014), which integrates new and existing R functions in order to perform basic population genetic analyses. I used default settings in PopGenReport to provide counts and frequencies of alleles, measures of genetic differentiation within and between populations, tests for null alleles, observed and expected heterozygosity, tests for departures from Hardy–Weinberg Equilibrium, pairwise genetic distances and tests for spatial autocorrelation. I also screened for null alleles, large allele dropout and scoring errors using the program MICRO-CHECKER v.2.2.3 (Van Oosterhout et al. 2004). Additionally, unbiased estimates of allelic richness and private alleles per sample location were calculated via rarefaction using the program HP-Rare 1.0 (Kalinowski 2005). Furthermore, I analyzed for linkage disequilibrium among loci using the program GENEPOP 4.2 (Raymond & Rousset 1995; Rousset 2008). Whenever multiple testing occurred, I used a Bonferroni adjusted p-value.

Population structure and statistical significance

Population structure was inferred using complementary approaches: Bayesian assignment in STRUCTURE (Pritchard et al. 2000) and discriminant analysis of principle components using DAPC within the *Adegenet* package (Jombart et al. 2010) in R. STRUCTURE was used to

estimate the number of genetic clusters apparent from the data using the log likelihood of individual assignment into K inferred genetic clusters. I used a burn-in length of 25,000 iterations preceding each MCMC simulation (100,000 iterations for K = 1 to 10, repeated 20 times for each value of K). The program STRUCTURE HARVESTER (Earl & VonHoldt 2011) was used to assess and visualize likelihood values, including L(K), L'(K), L''(K) and ΔK (Evanno et al. 2005), in order to detect the number of genetic clusters that best fit the data. The program CLUMPP (Jakobsson & Rosenberg 2007) was used to optimize STRUCTURE runs, and the program DISTRUCT (Rosenberg 2003) was used to visualize the final solution for the optimal number of genetic clusters. Furthermore, DAPC from the R package ADEGENET provided a complementary assessment of genetic structure, free from underlying assumptions regarding Hardy-Weinberg equilibrium or linkage disequilibrium.

I assessed significance among sample locations for individual assignment probabilities to genetic clusters from STRUCTURE output using permutation tests in R and obtained p-values and confidence intervals using the 'perm' R package (Fay & Shaw 2010). I created resampled distributions for each river's genetic clusters consisting of 9,999 mean values from randomly selected subsamples of n = 30 each. I used these distributions to test the null hypothesis of randomly distributed assignment probabilities among sampled locations at a significance level of 95 percent.

Trait variation and statistical analyses

I used adult PIT-tag movement data in conjunction with genetic assignment probabilities to assess a phenotypic trait, maximum displacement of adult grayling from overwintering sites, with regard to genotype. Using tag tracks acquired through PIT-tag antenna arrays, I examined movement for uniquely identified fish for which I had also collected neutral genetic information. In addition to movement data acquired though PIT-tag arrays, I also included fish in the analysis that were

captured and tagged at or beyond the KUP6 antenna, with capture location serving as distance of downstream displacement. Individuals and their genotypes were identified using PIT-tag information and assignment probabilities from STRUCTURE and DAPC output files. I performed a two-way analysis of variance (ANOVA) in R (R Core Team 2016) to test for differences in assignment probability using maximum displacement distance (km) and genetic clusters (K1, K2, K3) as factors in the analysis.

Results

Microsatellite Screening and summary statistics

I found no evidence for null alleles across sample locations and loci, except for Kup2 at *Tar*103 and CS at *Tar*100. I found no evidence for scoring errors or large allele drop-out across sample locations and loci. Although I found two loci in three separate populations that showed significant deviation from null expectations (K6 at *Tar*101, K8 at *Tar*110 and Oks2 at *Tar*101), all populations appeared to be in Hardy-Weinberg equilibrium. Additionally, I found evidence for linkage among four sets of loci, *Tar*106 and *Tar*101; *Tar*101 and *Tar*104; *Tar*106 and *Tar*110; and *Tar*105 and *Tar*115. However, analysis of the same set of loci using individuals from a larger geographic data set in my study area showed no indication of linkage for these genes (Golden et al. unpublished). Variation from population genetics null models and signs of linkage among loci likely indicate presence of multiple subpopulations within samples (Wahlund 1928; Nei & Li 1973). All loci were highly polymorphic with rarified allelic richness over loci ranging from 9 to 15 alleles (Table 1). Allelic richness per sample location, as well as private allele richness, observed and expected heterozygosity and inbreeding coefficient (F_{IS}) are summarized in Table 1.

Microgeographic differentiation and site specificity

Overall, pairwise F_{ST} (Table 2) values were low among comparisons within watersheds and higher for comparisons among watersheds (Table 2). Within the Kuparuk watershed, F_{ST} values ranged from 0.00 to 0.03, with greatest similarity between Kup6-YOY and the adult samples (Table 2). Within the Oksrukuyik watershed, F_{ST} values ranged from 0.00 to 0.07, with the Oks0-YOY showing the greatest similarity to the upper Oksrukuyik adult locations (CS and OC) and Oks3-YOY showing the greatest similarity to the lower Oksrukuyik adult locations (OC and LSag) (Table 2).

Results from clustering analyses, STRUCTURE and DAPC, both indicated significant genetic structure in the data (Figures 2 to 4). Mean natural log probability of the data from STRUCTURE suggested four to six genetic clusters might well characterize genetic structure in the data (Figure 2a), while Delta K suggested genetic structure at K = 4 (Figure 2b). Graphs of individual assignment probabilities to K = 4 genetic clusters showed differences in distribution of genetic clusters based on watershed (Kuparuk versus Oksrukuyik), location within watersheds, and life stage (YOY versus Adults) (Figure 3). The yellow and red genetic clusters dominated the Kuparuk watershed, with the red genetic cluster appearing almost exclusively in the YOY samples (Figure 3a). The Kuparuk YOY yellow and red genetic clusters showed significant non-random distribution at Kup6 and Kup8 sample locations (Table 3). Additionally, although cluster mixing occurred at upstream (Kup2) and downstream (Kup8) YOY locations, the yellow genetic cluster was most prevalent from the headwaters downstream to Kup6, while the red genetic cluster associated most clearly with the furthest downstream location at Kup8. The blue and pink genetic clusters dominated the Oksrukuyik watershed, with both genetic cluster appearing in both YOY and adult samples (Figure 3b). Similar to the Kuparuk watershed, the Oksrukuyik YOY blue and pink genetic clusters showed significant non-random distribution at all sample locations (Table 3), with the blue genetic cluster dominating the headwater locations, YOY: Oks0 & Oks2 and Adult: CS & OC, and the pink genetic cluster dominating the lower reaches, YOY: Oks3 and

Adult: LSag (Figure 3b).

Bayesian information criterion (BIC) from DAPC suggested that the data is best described by six genetic clusters (Figure 2c). Based on assignments of individuals to each genetic cluster, DAPC genetic clusters identified closely YOY sample location for both watersheds (Figure 4 and Table 4). The Kuparuk watershed comprised the yellow, orange and red genetic clusters, while the Oksrukuyik watershed consisted of the blue, purple and pink genetic clusters (Figure 4). Within the Kuparuk watershed, adults from all sample locations appeared to associate with both the orange and yellow genetic clusters. The yellow genetic cluster almost exclusively comprised the Kup6 YOY sample location, while the orange genetic cluster was found at both the Kup2 and Kup6 YOY sample locations. No such association existed between Kuparuk adult samples and the red genetic cluster found almost exclusively in YOY samples largely located at Kup8. Thus, the upper reaches of the Kuparuk watershed appeared to be dominated by the orange and yellow genetic clusters, while the lower reaches were dominated by the red genetic cluster. Within the Oksrukuyik watershed, the upper reaches appeared to be dominated by the blue genetic cluster, the lower reaches were dominated by the blue genetic cluster, and the middle reach associated with the purple genetic cluster. Similar to the STRUCTURE results, DAPC found that adult samples from the upper and middle reaches of the Oksrukuyik watershed (CS and OC) matched YOY genetics from the upper reaches (Oks0) and adult samples from the middle and lower reaches (OC and LSag) matched YOY genetics from the lower reaches (Oks3). I found no indication of adults associating with the purple genetic cluster found exclusively in YOY samples.

Adult movement patterns

Spring passive integrative transponder (PIT) tag data was difficult to attain due to complications

with the spring freshet, which lead to destruction of PIT-tag arrays. PIT-tag data from 2012 from the Kuparuk River, however, showed that movement of individual fish was bimodal (Figure 5). Approximately 2/3 of the spawning population remained close to the headwaters (GCL and Kup2) traveling 0 to 2 km from the overwintering location at GCL. The other 1/3 of the spawning population traveled 20 to 50 km downstream of the overwintering locations (Figure 5). In Oksrukuyik Creek, movement data for the spring was similarly difficult to attain. Data from 2016 indicated two distinct movement patterns, as well. I found upstream movement from the lower reaches near Oks3 toward the middle reaches near Oks1, but another group of individuals appeared to remain close to the headwaters, near Oks-1 (Figure 6).

Adult movement and microgeographic genetic differentiation

Data for genetically identified, PIT-tagged adult Arctic grayling were only available for the Kuparuk watershed and included tag detections from 2010 to 2013. Samples including both genetic and PIT-tag data totaled 48 individuals. PIT-tag antenna arrays showed variation in movement patterns through maximum displacement of individuals from the overwintering location, with maximum displacement ranging from 0 km to 74 km (Table 5).

Analysis of assignment probability to genetic clusters versus maximum displacement of individuals concurred with results from STRUCTURE and DAPC. Assignment probability to each of the Kuparuk genetic clusters was significantly associated with maximum displacement of individuals from the overwintering location (Figure 7, Table 6). I found significance among genetic clusters (p-value < 0.0001) and for the interaction between genetic cluster and displacement distance (p-value $= 0.041$). The yellow genetic cluster for PIT-tagged adults showed the highest assignment probabilities at a displacement of 8 km and lower assignment probabilities to the yellow genetic cluster beyond 8 km. Inversely, the orange genetic cluster for PIT-tagged

adults showed the highest assignment probabilities between 20 km and 38 km displacement from the headwaters. The red genetic cluster showed low assignment probabilities across all displacement distances from the headwater overwintering location.

Discussion

Despite difficulties associated with studying trait variation in long-lived species, I presented evidence suggesting that migration distance of spawning adults that originate from the same watershed corresponds strongly with microgeographic neutral genetic differentiation. Migration, homing and straying in salmonids is known to be highly polygenic, with many factors likely to influence spawning site fidelity. Thus, the exact mechanisms through which correlations arose remain unknown. Nevertheless, within both watersheds I discovered distinct headwater and downstream genotypes that appeared to express opposing migration strategies: downstream movement for headwater populations and upstream movement for downstream populations. Furthermore, within the Kuparuk watershed, downstream movement further differentiated into multiple spawning stocks based on migration distance. These watersheds were characterized by wide variation in aquatic habitat suitability due to drought-prone versus drought resistant river segments. Although not conclusive, one plausible explanation for the patterns observed in this study could be strong selection for migratory phenotypes that maximize Arctic grayling fitness in different locations within the watersheds.

Seasonal movement of fish into drought-prone river segments could alter population demographics and structure through reduced survival and recruitment. Past studies have documented effects of drought on demographics and persistence of fish populations (Cowx et al. 1984; Davies et al. 1988; Griswold et al. 1982). More recently, Penha et al. (2014) found that drought caused high mortality for the neotropical fish, *Hyphessonbrycon eques*, producing

changes in populations size structure, while floods yielded good recruitment and increased survival probability. Similarly, Nicola et al. (2009) found magnitude and duration of low flow events during the summer to be critical factors for young trout (*Salmo trutta*) survival and recruitment in Mediterranean streams. Additionally, White et al. (2016) discovered that fish survival during drought strongly depended upon refuge habitat size and the interplay between density dependent and density independent factors. During drought I have observed entrapment of Arctic grayling by dry river zones, which stranded fish in overcrowded, isolated pools, where resource limitation caused substantial weight loss by the end of the growing season. Rivers with dry zones, therefore, might comprise selectively variable environments with different survival probabilities depending on combinations of density independent factors, such as water temperature, oxygenation and flow, and density dependent factors resulting from reduced habitat size as wetted river area shrinks.

Drought-resistant river segments, on the contrary, might contribute to observed patterns of differentiation and migration by increasing survivorship of young and fitness of individuals. Migration locations and YOY genetic clusters in the Kuparuk River coincided with known areas of groundwater upwelling, which retain more constant water temperatures during the summer and resist freezing in winter compared to drought-prone river segments (Golden unpublished data). Heggenes et al. (2011) reported that groundwater may be important to salmonids for a number of reasons, including modulating temperatures, influencing water quality (such as nutrients and oxygen concentrations), providing river base flows, and providing refugia. For example, Saltveit and Brabrand (2013) found that groundwater increased survival of Atlantic salmon eggs in regulated streams, particularly during low flow periods. Examples of salmonid species that rely on groundwater exchange zones for spawning include brown trout, Arctic char, Atlantic salmon (Brabrand et al. 2002; Heggenes et al. 2011; Brabrand et al. 2006), brook trout (Curry & Noakes 1995), bull trout (Baxter & Hauer 2000), chum salmon (Mouw et al. 2013) and many more. Similarly, Arctic grayling might home to areas of groundwater discharge for spawning to enhance

survivorship of eggs and young. Why certain genotypes might prefer one upwelling location over another might relate to homing through olfaction, magnetic fields or other mechanisms (Keefer & Caudill 2014). Whatever the mechanism, groundwater upwelling at locations, such as Kup2, Kup6 and Kup8 could increase survivorship and fitness of individuals compared to other locations within the river, which are essential criterion for natural selection to act within a population.

If spawning site fidelity for Arctic grayling is heritable and expresses variability, it could be acted upon by natural selection to produce genetically different spawning stocks. A recent review on alternative migratory tactics for salmonids suggested the importance of additive genetic variation and spatial and temporal segregation of spawning activities as mechanisms for generating variation in migratory phenotypes (Dodson et al. 2013). Telemetry and tagging studies have shown strong site fidelity to spawning, feeding and overwintering locations by Arctic grayling (Blackman 2002; Buzby & Deegan 2000), including deep pools, spring-fed areas and lakes (West et al. 1992). High mortality of individuals or low spawning success within dry zones might select for migration tactics that avoid dry zones as spawning and rearing habitats, thereby segregating spawning activity. Boula et al. (2002), for example, found that significant genetic differentiation of sympatric resident and anadromous brook charr, *Salvelinus fontinalis*, in Quebec was related to the segregation of spawning sites. I found significant neutral genetic differences among YOY Arctic grayling sampling locations, providing evidence supporting sympatric genetic differentiation and suggesting isolated spawning activities. Although not conclusive, I speculate that variation in river drying and location of groundwater upwelling sites might promote spawning site segregation and selection for migratory genotypes through variation in survival across the aquatic landscape.

The neutral genetic microgeographic differentiation patterns found in this study could also be attributed to alternative explanations, such as river dry zones acting as barriers to

movement or to phenotypic plasticity of site fidelity. Effects of barriers on neutral genetic structure of river species have been well documented, particularly for dams and culverts (Junker et al. 2012; Peterson & Ardren 2009; Roberts et al. 2013) and for droughts (Fitzpatrick et al. 2014; Meeuwig et al. 2010; Hopken et al. 2013; Perkin et al. 2014) that physically reduce connectivity of the aquatic landscape. In fact, in chapter one of this dissertation I found that on a broad spatial scale adult Arctic grayling population structure strongly associated with river distance and dry river zones. Yet although dry zones exist within watersheds, spring spawning for Arctic grayling coincides with the Arctic spring freshet, when dry zones are bank-full and readily passable by fish. Because dry zones do not fragment the aquatic habitat until well after spawning activities cease, dispersal by Arctic grayling within watersheds should not be affected by dry zones as physical barriers. Location specific life-history factors, such as spawning site fidelity (Ozerov et al. 2012; Vähä et al. 2007; Fausch et al. 2002; Maria et al. 2012) have also been indicated in shaping genetic differentiation for stream fish. The mechanisms through which site fidelity arises in salmonids, however, appear complex and might involve polygenic interactions, for example, endocrine and neurological processes, as well as interaction with the environment, see Keefer & Caudill (2014) for a recent review on this topic. Although some site fidelity variation appears to have evolved in response to locally-adaptive selective pressures in salmonids (Hendry et al. 2004; Quinn 2005; Garcia de Leaniz et al. 2007), the genetic contribution to homing and straying remains an area of active research.

With climate change accentuation variability in habitat suitability across the globe, understanding adaptive potential of species and the traits upon which selection acts enhances our ability to predict species responses to environmental change. The interaction between species traits, the environment and the balance between selection and drift often lead to new phenotypes, presumably better suited to novel conditions. But, variability in the genome for traits under selection must persist for local adaptation to ensue. Despite current genomic advances, adaptive significance of trait variation in salmondis remains largely unknown (Garcia de Leaniz et al.

2007). The presence of microgeographic differentiation underscores the importance of trait variation in shaping fine-scale population structure and species evolution and persistence. This study presents an initial investigation of a potentially significant trait for Arctic grayling persistence in a rapidly changing aquatic landscape.

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Figures

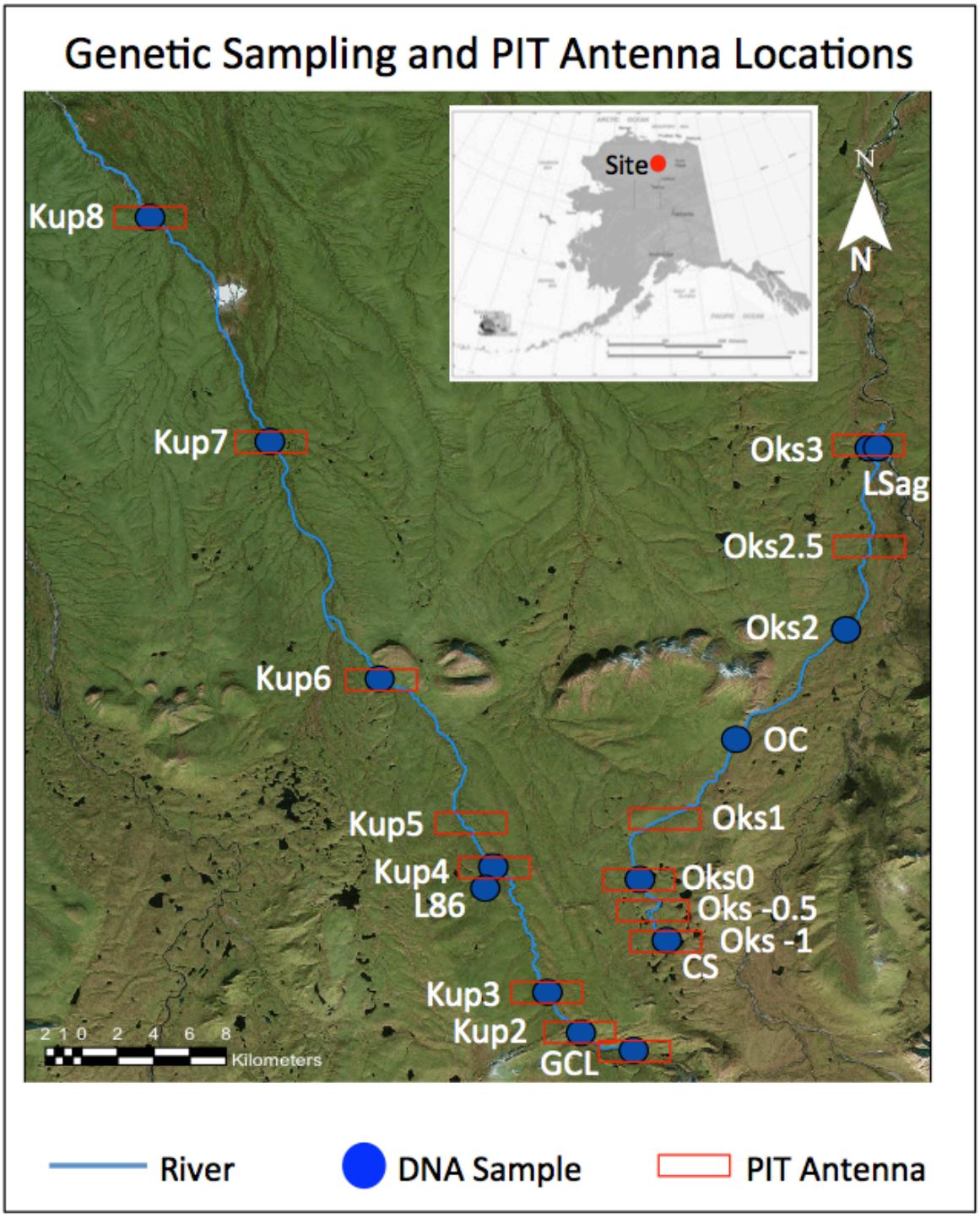


Figure 1. Study area and sampling locations. Blue circles represent genetic sampling locations. Red rectangles indicate PIT-tag antenna arrays.

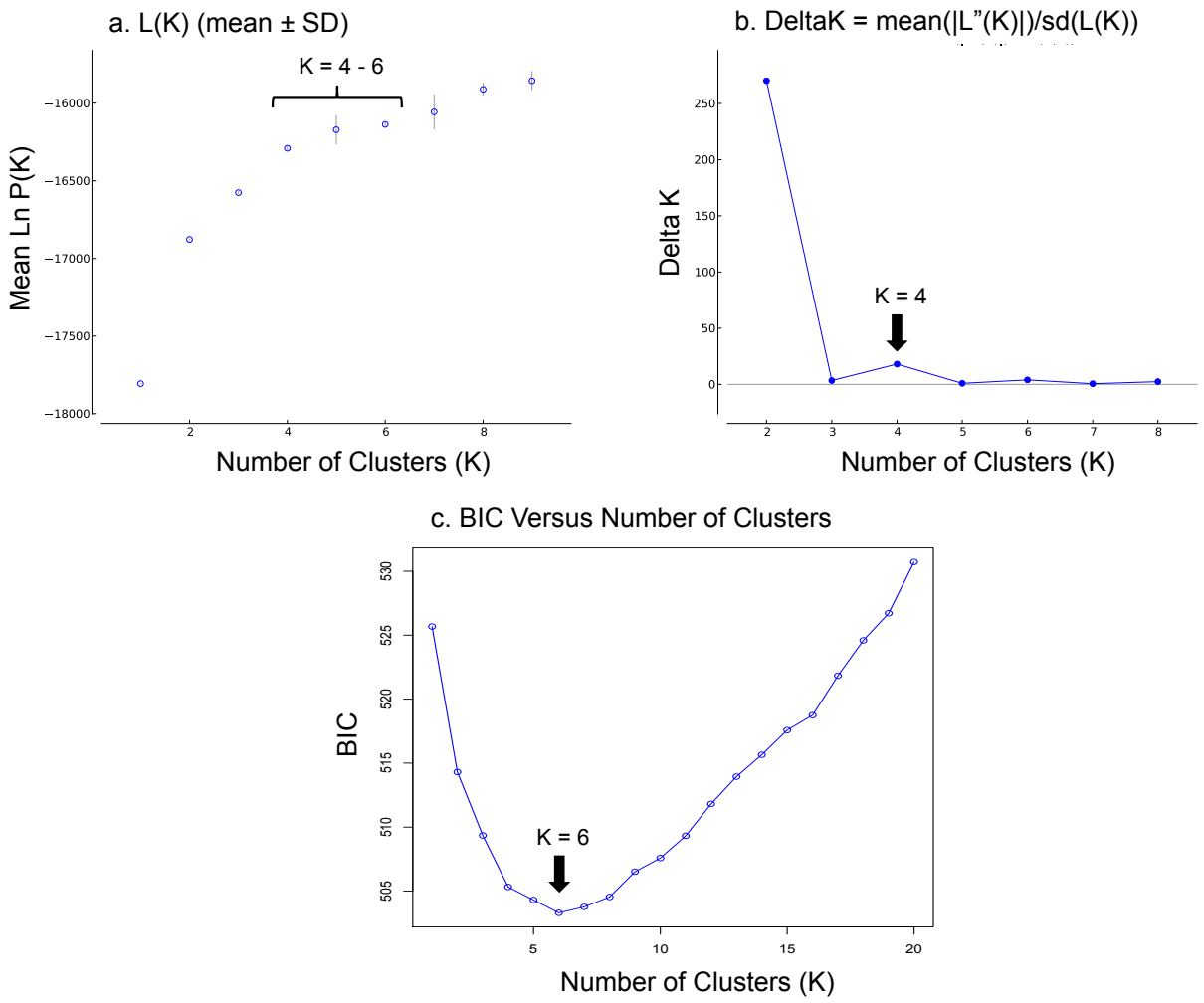
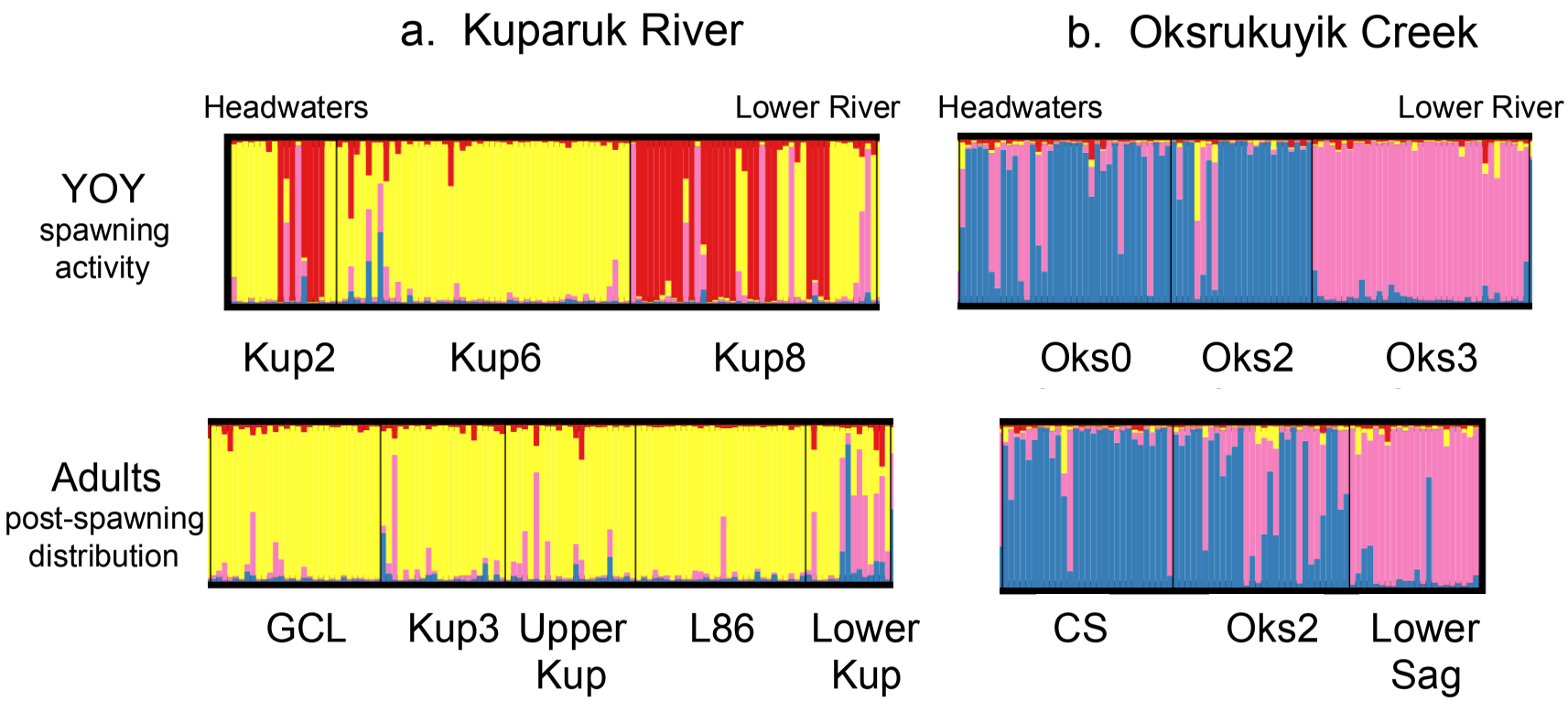


Figure 2. Clustering results from STRUCTURE (a and b) and DAPC (c) analyses. Most likely values for genetic cluster are indicated with arrows.

Figure 3. STRUCTURE plots for K=4 genetic clusters. Vertical bars represent individual assignment probabilities to each of four genetic clusters (yellow, red, blue and pink) for the Kuparuk watershed (a) and Oksrukuyik watershed (b). Sampling locations are indicated along the x-axis for both YOY and Adult Arctic grayling.



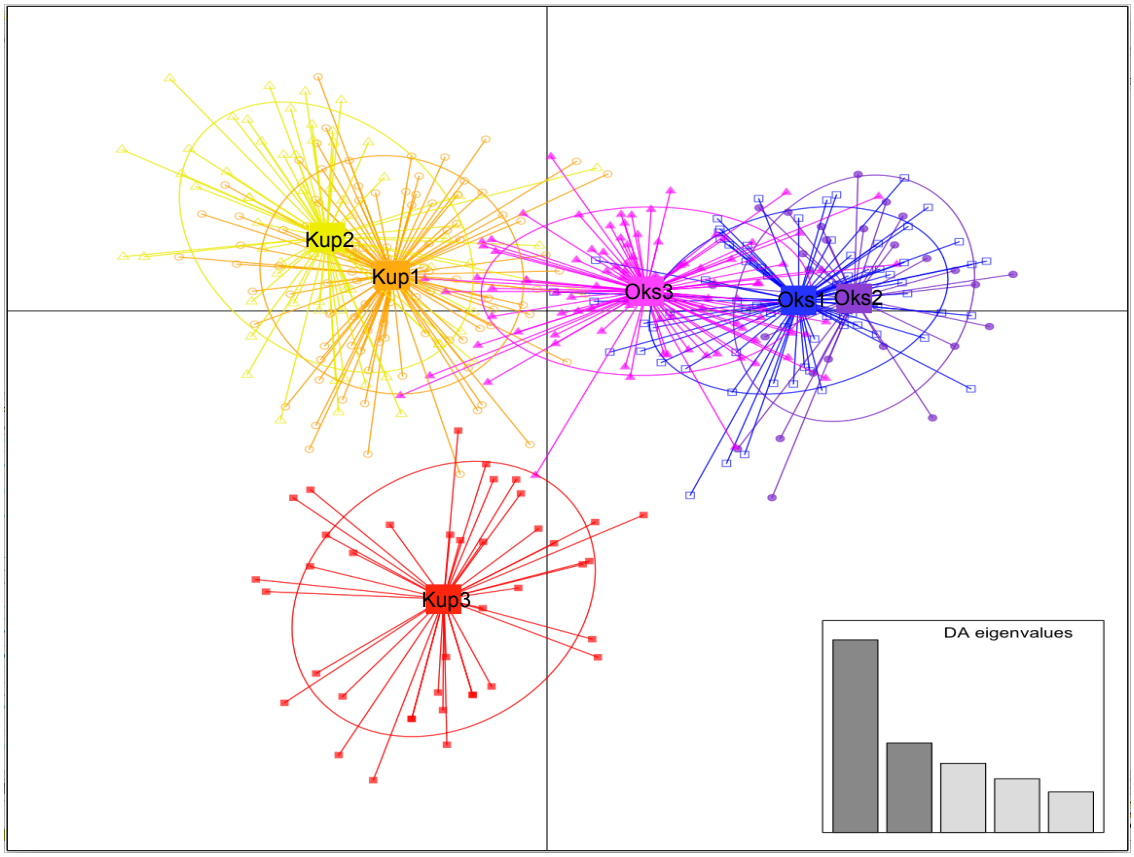


Figure 4. DAPC cluster results showing the first two eigenvalues. Genetic clusters associated with the Kuparuk watershed are indicated by orange (Kup1), yellow (Kup2) and red (Kup3) [Whoops! Need to fix those.]. Genetic clusters associated with the Oksrukuyik watershed are indicated by blue (Oks1), purple (Oks2) and pink (Oks3).

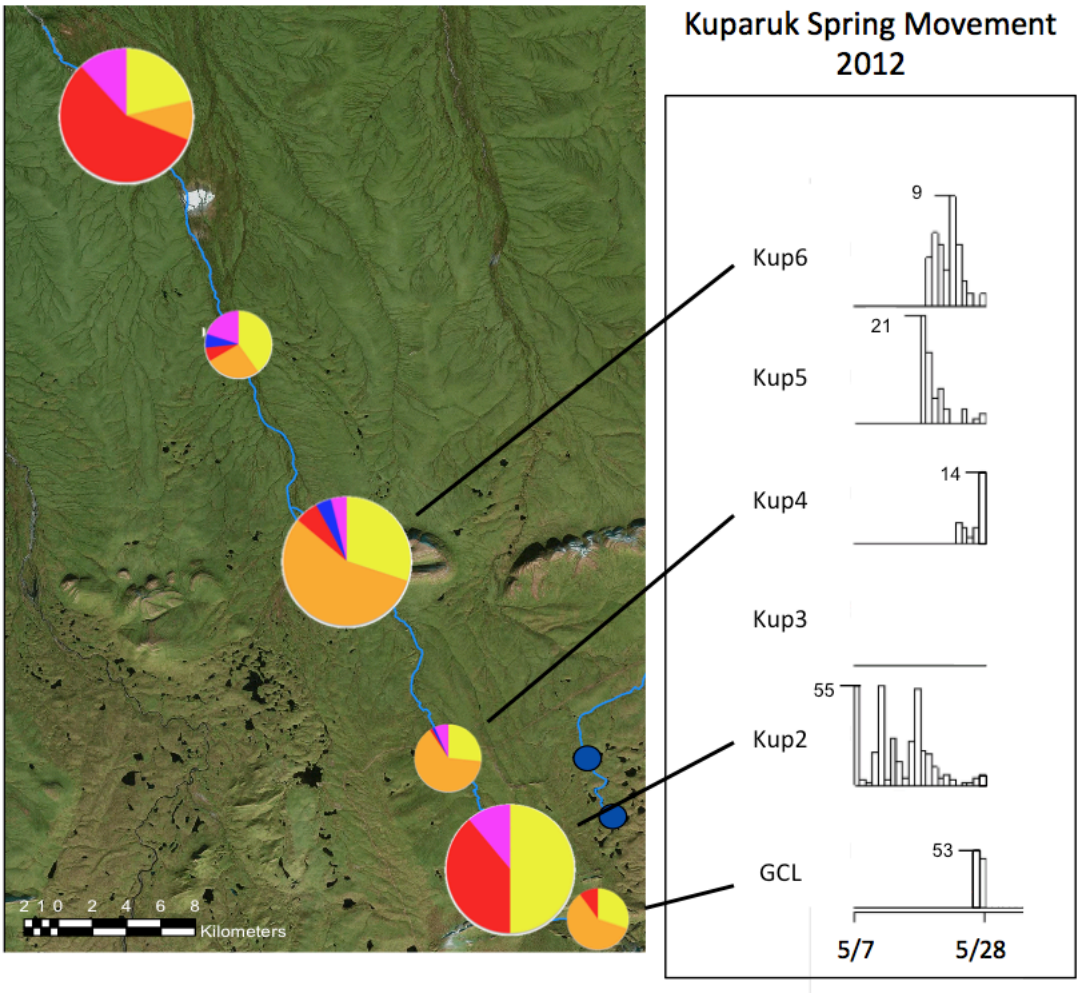


Figure 5. Kuparuk River YOY and adult grayling percent assignments and adult PIT-tag movement patterns. Percent assignments of individuals to DAPC genetic clusters (see Figure 4). YOY genetic assignments are indicated with large circles and adult genetic assignments are indicated with small circles. PIT-tagged adult Arctic grayling spring movement patterns for 2012 are indicated by histograms of detections at PIT-tag antenna arrays. Date of detection is indicated along the x-axis. Numbers on histogram bars indicate scale of the y-axis in number of individuals. Antenna arrays from the headwaters at GCL to Kup6, approximately 50 km downstream, appear along the y-axis.

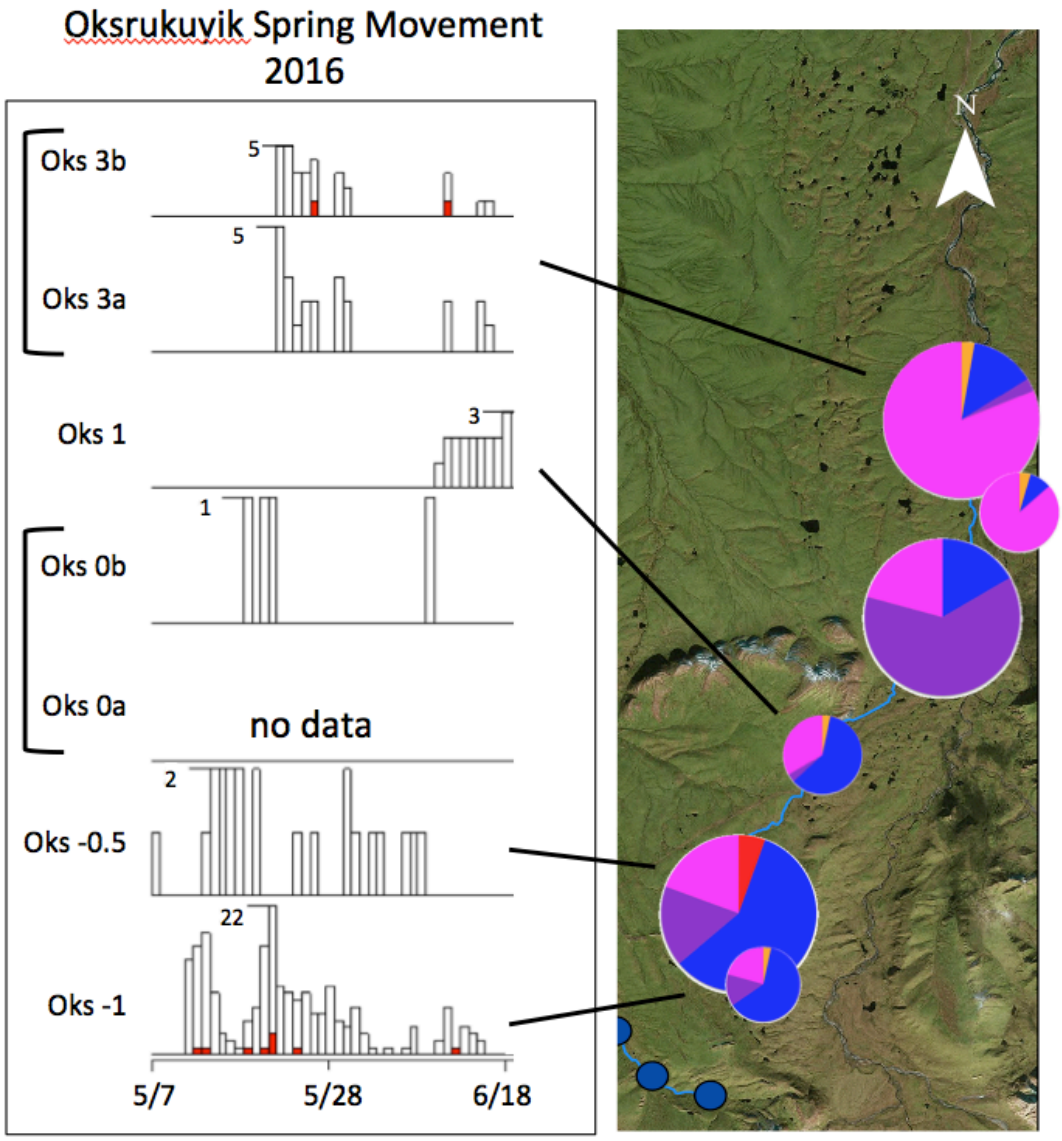


Figure 6. Oksrukuyik Creek YOY and adult grayling percent assignments and adult PIT-tag movement patterns. Percent assignments of individuals to DAPC genetic clusters (see Figure 4). YOY genetic assignments are indicated with large circles and adult genetic assignments are indicated with small circles. PIT-tagged adult Arctic grayling spring movement patterns for 2016 are indicated by histograms of detections at PIT-tag antenna arrays. Date of detection is indicated along the x-axis. Numbers on histogram bars indicate scale of the y-axis in number of individuals. Antenna arrays from the headwaters at Oks -1 to Oks3, approximately 50 km downstream, appear along the y-axis. Last detection indicated by red.

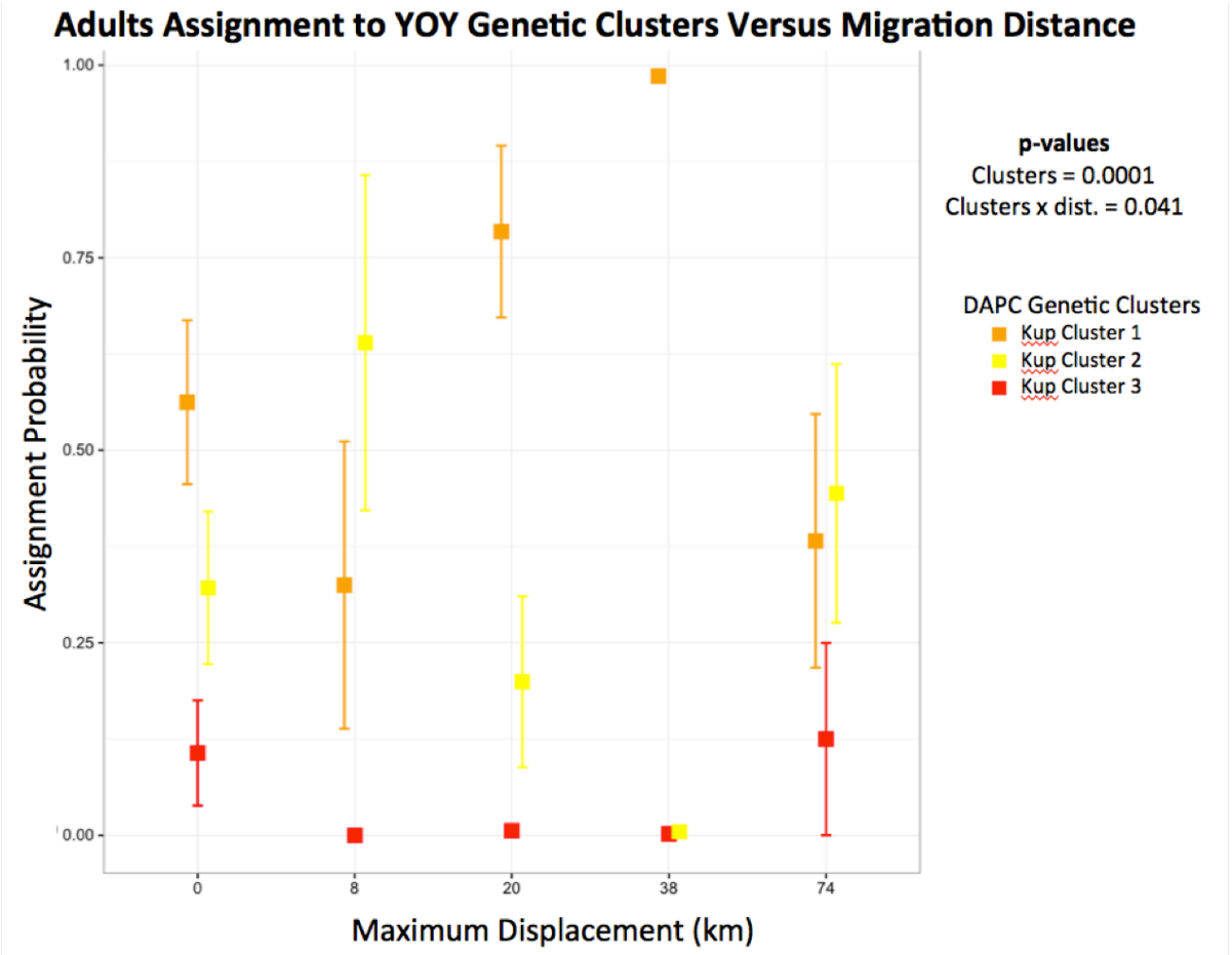


Figure 7. Assignment probability versus maximum displacement (km) for PIT-tagged adult Arctic grayling in the Kuparuk watershed. Genetic clusters to which individuals are assigned appear are indicated by color: orange = Kup2, yellow = Kup6, red = Kup8.

Tables

Table 1. Microsatellite summary statistics. Ar – Alleleic richness and Pr – private allele richness (*rarified to 30 genes), observed and expected heterozygosity (H_o and H_e), and inbreeding coefficient (F_{IS}) for YOY and adult sampling locations within the Kuparuk and Oksrukuyik watersheds.

River	Stage	Location	A_r*	PrA_r*	H_o	H_e	F_{IS}
Kuparuk	YOY	Kup2	10	0.05	0.84	0.87	0.03
		Kup6	11	0.17	0.85	0.87	0.03
		Kup8	10	0.09	0.88	0.86	-0.01
	Adults	GCL	11	0.02	0.86	0.88	0.02
		KUS	12	0.24	0.87	0.90	0.03
		Kup4	12	0.47	0.90	0.89	0.00
		L86	11	0.13	0.86	0.88	0.02
		Kup7	13	0.50	0.87	0.89	0.02
Oksrukuyik	YOY	Oks0	10	0.36	0.86	0.87	0.01
		Oks2	9	0.00	0.78	0.77	-0.01
		Oks3	13	0.62	0.88	0.91	0.02
	Adults	CS	10	0.23	0.80	0.85	0.06
		OC	11	0.19	0.91	0.89	-0.02
		LSag	13	0.63	0.93	0.92	-0.01
	All	Mean	11.18	0.26	0.86	0.87	0.01
		St Dev	1.26	0.21	0.04	0.03	0.02

Table 2. Pairwise Nei's F_{ST} for YOY and adult sample locations within the Kuparuk and Oksrukuyik watersheds.

		Kuparuk River								Oksrukuyik Creek					
Location	Sample	Kup2-YOY	Kup6-YOY	Kup8-YOY	GCL	KUS	Kup4	L86	Kup7	Oks0-YOY	Oks2-YOY	Oks3-YOY	CS	OC	LSag
Kuparuk River	Kup2-YOY	0.00													
	Kup6-YOY	0.02	0.00												
	Kup8-YOY	**0.01**	0.02	0.00											
	GCL	0.02	**0.01**	0.02	0.00										
	KUS	0.02	**0.01**	0.02	**0.01**	0.00									
	Kup4	0.03	**0.01**	0.02	**0.01**	**0.01**	0.00								
	L86	0.02	**0.01**	0.02	**0.01**	**0.01**	**0.01**	0.00							
	Kup7	0.03	**0.01**	0.02	0.02	0.02	0.02	0.02	0.00						
Oksrukuyik Creek	Oks0-YOY	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.00					
	Oks2-YOY	0.08	0.07	0.06	0.07	0.07	0.07	0.07	0.07	0.03	0.00				
	Oks3-YOY	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.06	0.00			
	CS	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	**0.02**	0.05	0.03	0.00		
	OC	0.04	0.03	0.03	0.03	0.03	0.03	0.04	0.04	**0.02**	0.05	**0.02**	**0.02**	0.00	
	LSag	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.07	**0.02**	0.03	**0.02**	0.00

Table 3. Permutation tests of significance comparing YOY assignment probabilities at sampling locations to null distributions for each genetic cluster.

River	Cluster	Distribution	mean	lower CI	upper CI	p-value
Kuparuk	Yellow	Null	0.6008	0.5997	0.6019	
		Kup2	0.5495	0.4378	0.6612	0.740
		Kup6	0.8890	0.8450	0.9331	0.002
		Kup8	0.2819	0.1872	0.3767	0.002
	Red	Null	0.2800	0.2790	0.2811	
		Kup2	0.3331	0.2273	0.4388	0.676
		Kup6	0.0405	0.0200	0.0610	0.002
		Kup8	0.5438	0.4357	0.6519	0.002
Oksrukuyik	Blue	Null	0.5009	0.5002	0.5016	
		Oks0	0.7455	0.6513	0.8397	0.004
		Oks2	0.8411	0.7361	0.9460	0.002
		Oks3	0.0422	0.0268	0.0576	0.002
	Pink	Null	0.4598	0.4591	0.4605	
		Oks0	0.2198	0.1283	0.3113	0.004
		Oks2	0.1129	0.0307	0.1951	0.002
		Oks3	0.9180	0.8956	0.9405	0.002

Table 4. Individual assignments to six DAPC genetic clusters (K1, K2, K3, Ok1, Ok2 and Ok3) for YOY and adult sampling locations within the Kuparuk and Oksrukuyik watersheds.

	Genetic Cluster					
Pop	**K1**	**K2**	**K3**	**Ok1**	**Ok2**	**Ok3**
Kup2 YOY	0	9	7	0	0	2
Kup6 YOY	28	15	3	2	0	2
Kup8 YOY	4	9	24	0	0	5
GCL	18	9	3	0	0	0
Kup3	12	9	0	0	0	1
Kup4	16	5	0	0	0	2
L86	20	6	1	1	0	2
Kup6	4	6	1	1	0	3
Oks0 YOY	0	0	2	21	6	7
Oks2 YOY	0	0	0	4	15	5
Oks3 YOY	1	0	0	5	1	30
CS	1	0	0	18	4	6
OC	1	0	0	18	1	10
LS	1	0	0	2	0	19
N	106	68	41	72	27	94

Table 5. PIT-tagged adult grayling antenna locations, number of individuals at each location and distance form the headwaters for each location.

Location	N	Displacement (km)
GCL	20	0
Kup3	3	8
Kup4	11	20
Kup6	5	38
Kup8	8	74

Chapter 3:

Habitat fragmentation alters vital rates for an iteroparous freshwater fish

Abstract

The need to predict species persistence under climate change scenarios necessitates an understanding of the effects of environmental change on population vital rates. This study investigates the effects of drought-induced aquatic habitat fragmentation on vital rates and movement patterns of Arctic grayling (*Thymallus arcticus*) in tundra streams using indications of body condition, histological analyses and remote sensing of uniquely tagged individuals. I found that entrapment due to drought decreased fish mass by an average of 30 grams during the 2011 fall migration. However, I found no significant differences in body condition, rates of oocyte atresia, or spawning movement patterns following drought (spring 2012), suggesting that Arctic grayling do not skip spawning in response to poor pre-spawning conditions. PIT-tag antenna surveys revealed highly significant differences in post-spawning movement patterns between trapped and non-trapped individuals, suggesting high post-spawning mortality due to entrapment by drought. This study underscores the importance of understanding trade-offs between individual survival and fecundity for predicting the affects of altered hydrology on population persistence in a region undergoing rapid climate change.

Introduction

The Arctic is warming rapidly in response to climate change with unknown consequences for population dynamics and persistence. In some areas of the Arctic, changes in hydrology have caused increased river drying and aquatic habitat fragmentation (Betts & Kane 2010). Climate warming exacerbates drought in tundra streams when evapotranspiration rates exceed precipitation rates during the summer growing season (Kane et al. 2004; Hinzman et al. 2005). Arctic freshwater species often require access among habitats to fulfill life history requirements, such as spawning migrations, yet dendritic stream networks

provide few alternatives to entrapment when fragmentation occurs (Fagan 2002). In order to optimize use of stream habitat, Arctic fish must time movements to maximize access to stream resources, while avoiding freezing in winter (Power & Reynolds 1997). Therefore, the effects of climate induced habitat fragmentation on population demographics in these systems could be severe.

The relationships between resource acquisition and allocation toward growth, survival and fecundity are complex and often involve trade-offs between individuals and their progeny (Rideout et al. 2005). For fish, these trade-offs fall along a continuum. At one extreme, semelparous species spawn only once within a lifetime, with the energetic cost of reproduction exceeding that attained through lipid storage, ultimately sacrificing longevity for fecundity (Hendry & Berg 1999). Iteroparous species that spawn repeatedly within a lifetime must strike a balance between individual condition and reproductive output. For example, the prevalence of skipped-spawning, where individuals in poor condition temporarily forgo seasonal reproduction, suggests a trade-off between growth, maturation, and fecundity (i.e. Trotter et al. 2012; Sitar et al. 2014; Kennedy et al. 2011).

Environmental conditions leading up to spawning could affect individual condition, thereby altering spawning strategies in fish. Pecquerie et al. (2009) demonstrated that for anchovy, conditions encountered prior to spawning altered their ability to build up reserves allocated to reproduction. Pangle (2004) found that decreased energy stores increased mortality of small herring and partly explained variability in recruitment. Similarly, Cargnelli and Neff (2006) found that both energetic (adult condition) and behavioral (timing of spawning) factors play a role in determining reproductive strategy and potential in bluegill sunfish.

In the Arctic, fish body condition at the end of the growing season might play a critical role in population demographics due to the limited seasonal availability of resources. Habitat fragmentation might alter synchronization between resource acquisition and growth, survival and reproduction (Marchand 1996), thereby altering vital rates, such as fecundity (Ganias 2013). Changes in watershed hydrology that reduce aquatic connectivity could delay access to critical habitats during key life history

events (Betts & Kane 2015), such as migration to overwintering or spawning locations, thereby influencing population vital rates.

Here I examine the effects of drought on vital rates and movement patterns of Arctic grayling in tundra streams, by comparing fish condition, histology of oocytes, and individual movement patterns between fish trapped by drought and fish that avoided entrapment. I hypothesized that individuals that experienced delayed fall migration due to drought would show decreased condition, reduced fecundity, and altered spring movement patterns the following spring compared to non-trapped individuals. Using passive integrated transponder (PIT) tagged adults to identify trapped versus non-trapped individuals, histological analysis of oocytes and analysis of PIT-tag antenna movement data, I investigated the effects of drought on Arctic grayling vital rates and movement patterns in the Kuparuk River, Alaska. I predicted that reduced aquatic connectivity between summer and winter habitats would influence Arctic grayling survival and reproduction by decreasing stored energy necessary for overwinter survival and spawning capability. In particular, I predicted that (1) trapped individuals would show increased rates of oocyte atresia (Lubzens et al. 2010) when compared to non-trapped individuals; (2) trapped individuals would alter spring movement patterns by skipped-spawning compared to non-trapped individuals; and (3) post-overwinter survival and condition of trapped individuals would be reduced compared to non-trapped individuals.

Methods

Study area, background and natural history

This study was conducted in the Kuparuk River located on the North Slope of the Brooks Mountain Range, Alaska (Figure 1a). The Kuparuk River is a clear water Arctic tundra stream consisting of alternating pool, run and riffle habitat. The river flows during the Arctic growing season from May to late September and freezes solid from mid-September to early May. Because few overwintering habitats exist in this system, fish inhabiting the stream during the open-water season must rely on an interconnected aquatic landscape to access suitable spawning, feeding and overwintering locations.

Sections of the Kuparuk River, however, are susceptible to drought, with large stretches of river drying occurring in years when evapotranspiration exceeds precipitation (Kane et al. 2004). When dry river zones occur, these areas are impassible and uninhabitable by fish occupying tundra streams during the summer growing season (Betts & Kane 2015).

The highly migratory salmonid, Arctic grayling, is the only fish species commonly found within streams in this study area. Adult Arctic grayling tend to occupy deep, fast-moving water in pools and runs, whereas age-0 and juvenile grayling occupy shallow, low-current side and backwater areas of the rivers. Adult grayling in the Kuparuk River overwinter in the headwater lake and make an annual spawning migration in the spring after ice-out occurs to locations up to over 70 km downstream of the headwaters. In August 2011, a drought occurred during the Arctic grayling fall migration in the Kuparuk River (Figure 1b), detaining a large subset of the migratory population (Figure 1c) and delaying arrival at the overwintering site (Figure 2). This drought and concurrent entrapment provided an opportunity to investigate the effects of river drying on Arctic grayling survival and vital rates by tracking and assessing condition of non-trapped and trapped PIT-tagged individuals over time.

PIT-tag antenna arrays

I established and maintained a series of stationary PIT-TAGantenna arrays from the headwaters of the Kuparuk River to over 74 km downstream (Figure 1a). Each PIT-TAGantenna station consisted of an antenna, a tuner box, a marker tag, an Oregon RFID reader and a power station (Figure 3). The antenna consisted of a loop of antenna wire placed in cross-section within the stream channel that connected to a tuner box, which tuned the antenna to half-duplex PIT-tag frequency. The antenna and reader box were powered by three six-volt, deep-cycle, lithium batteries and recharged using solar panels. A marker tag was used to indicate when PIT-tag antennas ceased functioning due to environmental condition or power shortages. PIT-tag antenna arrays were deployed during the ice-free season (late May to mid-September) from 2010 to 2013.

Fish Sampling

In addition to ongoing PIT-tagging efforts by the Arctic Long Term Ecological Research (LTER) project within the Kuparuk River from July to September 2011, I sampled adult Arctic grayling from late May to early June 2012, as they emerged from their overwintering location (GCL, Figure 1a). Arctic grayling were captured during the summer growing season using fyke nets and by angling and during the spring migration using a weir trap situated at the outlet of the overwintering lake. I identified two subsets of individuals in 2012 using PIT-tags: (1) fish that entered the overwintering site prior to the 2011 drought (non-trapped) and (2) fish trapped in the KUS pool in 2011 prior to migrating to the overwintering site (trapped). Fish caught in 2011 and fish that were not part of the two groups (trapped and non-trapped) were anaesthetized in a eugenol solution (50 mg/L Aqui-SE®), measured to the nearest 0.1 cm, weighed to the nearest 0.1 gram, and PIT-tagged. I derived fish condition using regression residuals from the linear model of log(weight) versus log(length). I tagged fish using half-duplex, 23-mm PIT-tags by making a small incision on the ventral side of the fish below the pelvic girdle and inserting the tag into the body cavity using a syringe. Once fully recovered, tagged fish were released back to the river near their sampling location. A subset of the trapped and non-trapped fish was captured in the spring of 2012 during the spawning migration and euthanized for further analyses (see below). The remaining fish were tracked via PIT-tag antennas from spring 2012 to fall 2014.

Fecundity and histology of oocytes

I collected and analyzed gonad tissue to evaluate spawning status and fecundity for trapped versus non-trapped fish. Fish were euthanized using overexposure in eugenol solution until respiration and responsiveness to external stimuli ceased. Each fish was then weighed and measured as above. I removed female gonads *en masse* and liver through surgical dissection and weighed each tissue to the nearest 0.1 gram. I weighed a subset of oocytes from each fish and counted the number of maturing oocytes within

each subset in order to estimate total and relative fecundity for each female (Moyle & Cech 1996). I fixed the remaining gonad tissue in 10% buffered formalin solution, covering each gonad with at least 10x the volume of tissue. Later, preserved gonads were later, sectioned to approximately 1 cm thick, placed in cassettes and submitted to the University of Connecticut's Pathobiology Department, where they were processed (dehydrated, embedded in paraffin, hematoxylin and eosin stained and thin-sectioned) to produce histological slides. I created slides for anterior, central and posterior portions of each ovary to ensure oocytes were distributed homogenously within the ovaries (Mumford et al. 2007). I examined histological slides to assess percent oocyte developmental stage and used these values to estimate gonad phase (Brown-Peterson et al. 2011).

I evaluated histological slides for oocyte stage under a compound microscope. Because histological criteria for Arctic grayling gonads are lacking, I created criteria for determining oocyte stages and ovarian phases using guidelines from Brown-Peterson et al. (2011) for standardizing terminology of reproductive biology in fishes (Figure 4; Table 1a and b). For each histological slide, I determined oocyte stages (Table 1a) within five randomly selected grid cells (1 cm^2 each) placed on top of the histology slide and calculated the total number of each type of oocyte present. Presence, abundance and proportion of different oocyte stages determined ovary phase (Table 1b).

I determined fecundity by weighing a subsample of oocytes in the field from each individual's ovaries (described above), counting the number of vitellogenic oocytes within the subsample and calculating mass (g) per oocyte. I then extrapolated to total fecundity by multiplying gonad mass by number of oocytes per gram to attain total number of ooctyes per female. Relative fecundity was calculated by dividing each female's fecundity by the total wet weight of the female.

Gonadosomatic and hepatosomatic indices

Gonadosomatic index is defined as the proportion of body mass apportioned to gonads and provides an indication of resources allocated to reproduction (Barber & Blake 2006). Similarly, hepatosomatic is

defined as the proportion of body mass allocated to the liver and indicates the status of energy reserves (Diana 1995). Because fish often store energy as lipids in their liver, fish with low reserves tend to have lighter livers. Both indices provide general health and condition information, which I used to compare between trapped and non-trapped individuals. I collected and weighed whole gonad tissue to the nearest 0.1 gram and derived gonadosomatic index (GSI) by dividing wet weight of each fish's gonad by wet weight of the fish. Similarly, I weighed liver tissue to the nearest 0.1 gram and calculated hepatosomatic index (HSI) by dividing wet weight of each fish's liver by wet weight of the fish.

Statistical analyses

Due to small sample size and assumptions regarding normality, I used permutation tests constructed in R (R Core Team 2016) instead of ANOVA to compare difference found among trapped and non-trapped individuals. I compared the sample's test statistic to the sampling distribution of the test statistic acquired when the null hypothesis was true. I created null distributions by shuffling the group labels (non-trapped and trapped) within each data set and sampling the test statistic from the random data set 9999 times. I acquired p-values for each test by ranking the real test statistic among the shuffled test statistics and finding the probability that the test statistic was at least as extreme as observed if the null hypotheses were true. I conducted permutation tests for mean difference among non-trapped and trapped individuals for length (cm), weight (g), condition (residuals from the regression model of log(weight) versus log(length)), gonadosomatic index (GSI), hepatosomatic index (HSI) and relative fecundity.

In order to retain the use the detailed information on oocyte stages of individuals, I chose a multivariate ordination approach to examine ovarian differences among individuals instead of comparing ovary phases. I used constrained redundancy analysis with automatic selection of variables in the 'vegan' package (Oksanen et al. 2013) in R to examine oocyte stage with regard to fish length, weight, condition and group (non-trapped and trapped). Variables were selected using permutation p-values and a stepwise model selection procedure that included both forward and backward search modes. Terms were added to

the model for $p \leq 0.05$ and dropped from the model for $p > 0.1$. I chose 100 permutations per step, 1000 maximum ANOVA permutation steps and 50 iteration steps for dropping or adding terms.

PIT-tag antenna data were compiled into a single file and cleaned to remove marker tags, test tags and individuals repeatedly detected at a station on the same day. I coded the data either yes = 1 or no = 0 for individuals detected either in the overwintering lake or in the river during key seasonal movement phases (i.e. spawning in the river and overwintering in the lake). I assessed spring 2012 migration by determining number of unique PIT-tag detections within the river in 2012 after overwintering in the lake. I established fall 2012 return migration status by determining the number of unique PIT-tag detections found within the overwintering lake given that a fish had migrated in 2012. And, I assessed whether or not individuals were detected either within the overwintering lake or within the river in 2013 and again in 2014. I used generalized linear models with binomial distribution and a logit link using the 'stats' package in R. Significance of model parameters were assessed for $p \leq 0.05$.

Results

Length, weight and condition among groups

While detained in the river during the 2011 fall migration, Arctic grayling lost weight and condition (Fulton's K: $100W/L^3$; W = weight in grams and L = length in cm) compared to summer baseline data (Figure 5a & b). On average, trapped fish lost approximately 30 grams of mass while detained. Out of 286 individuals identified as trapped and 961 individuals identified as non-trapped, I recaptured 16 trapped individuals (10 female, 6 male) and 24 non-trapped individuals (6 female, 18 male) during the spring 2012 spawning migration (Table 2). Comparisons of body composition measurements showed no differences between trapped and non-trapped Arctic grayling for length, weight, condition or hepatosomatic index when males and females were analyzed together. Trapped females, however, showed significantly greater length, weight and gonadosomatic index compared to non-trapped females, but showed no significant differences in condition, hepatosomatic index or relative fecundity between groups (Figure 6, Table 2).

Fecundity and histology of oocytes

Despite having lost over 30 grams on average while trapped in the KUS pool during the drought of 2011, trapped fish showed no indication of skipped-spawning during the next breeding season through resorption of oocytes. My histological classification of oocytes revealed stages from primary growth through germinal vessical breakdown with little indication of post-ovulatory follicles or atresia (Table 3). A conspicuous lack of stage-one vitellogenesis oocytes in the samples indicated that Arctic grayling in the Kuparuk River deterministically spawn, producing a single batch of mature oocytes per spawning season (Brown-Peterson et al. 2011). Based on redundancy analysis of oocyte stages, I found no significant associations between oocyte stage and individual length, weight, condition or group (non-trapped, trapped) (Figure 7). Based on presence and percent of oocyte stages, most of the ovaries examined were in the actively spawning phase with a few in the spawning capable phase and only one in the developing phase (Figure 8; Table 3).

PIT-tag movement patterns

Based on a generalized linear model, I found highly significant differences for fish movement patterns following the drought among groups (non-trapped and trapped; p-value < 0.001) and between years (2012 and 2013; $p < 0.00001$). Out of 961 non-trapped and 286 trapped fish that entered the overwintering lake in the fall of 2011, I detected 243 non-trapped (25%) and 144 trapped (50%) individuals within the river the following spring. Based on permutation tests, this difference was not significant (p-value = 0.3998). I found a highly significant difference between groups, however, in the number of individuals returning to the overwintering lake in the fall of 2012 (p-value < 0.0001) (Figure 9). Of the fish that left the lake to spawn, 21 fish from the non-trapped group were detected returning to the overwintering lake in the fall of 2012, while none of the trapped fish were detected returning after leaving the lake in that spring (Figure 9). I followed the non-trapped and trapped PIT-tagged fish through subsequent years and found 109 non-

trapped fish in 2013 and 31 non-trapped fish in 2015 among the PIT-tag detection data, but none of the trapped fish were ever detected again (Table 4).

Discussion

I examined the effects of drought on vital rates and movement patterns of an Arctic freshwater fish, the Arctic grayling. Because drought caused pre-spawning weight and condition loss while fish were trapped, I predicted that trapped fish would show increased rates of atresia and altered movement patterns indicating skipped-spawning behavior. The results were surprising because I found little evidence of atresia and no significant difference in spring spawning movement patterns between trapped and non-trapped individuals. Analysis of subsequent movement patterns, however, indicated high post-spawning mortality for trapped individuals. Therefore, entrapment by aquatic habitat fragmentation greatly altered vital rates and movement patterns as hypothesized, but not consistent with initial predictions.

Poor pre-spawning condition due to harsh environmental conditions often leads to reallocation of resources, such that fish use resources initially allocated for reproduction toward survival instead (Jørgensen et al. 2006). Low nutritional condition prior to spawning is a main cause for ovarian atresia or egg resorption in iteroparous fish (Jakobsen et al. 2009). Thus, atresia can be viewed as a regulating factor between energy accumulated during the feeding period prior to spawning and the number of oocytes likely to successfully complete maturation during spawning. Previous work on Arctic grayling indicates that growth correlates with river discharge due to changes in food availability and metabolic demands as stream area, fish density, and river temperature fluctuate (Deegan et al. 1999; Deegan et al. 1997). Yet despite having lost over 30 grams on average while trapped in the KUS pool during the drought of 2011, trapped fish showed little to no atresia or pre-spawning differences in condition compared to non-trapped fish. Without data to compare condition of non-trapped fish during the 2011 drought, I do not know if all fish experience the same magnitude of end-of-season weight loss that trapped fish sustained. However,

Deegan et al. (1997) experimentally demonstrated density dependence for Arctic grayling documenting reduced lipid storage, hepatosomatic index and gonad mass at high fish density compared to average density in both high and low productivity environments. Perhaps, competition among individuals while trapped allowed some fish to capitalize on the limited resources available in the pool, while others were out-competed, thereby allowing the most competitive individuals to attain spawning capability. Differences in competitive ability among trapped individuals might help explain why I found no differences in relative fecundity between trapped and non-trapped fish and no difference in spring spawning movement patterns. These results suggested that instead of skipped-spawning, at least some trapped Arctic grayling acquired sufficient recourses, despite the overall trapped population's poor pre-spawning condition, to allocate toward egg production and spawning activities.

Although I did not observe the expected outcomes associated with pre-spawning resource limitation, post-spawning movement patterns revealed the most striking result of this study. I found high post-spawning mortality of trapped fish, suggesting that trapped grayling might have allocated energy stores other than lipids, such as from viscera, liver and muscle tissues, to complete gonad maturation and spawning activities. This behavior is common for semelparous species, such as sockeye salmon, but contrary to expectations for iteroparity. For example, Idler & Clemens (1995)found that sockeye salmon females used 41% and males used 30% of their carcass protein during spawning migration. Similarly, if entrapment and loss of body condition during the previous year's fall migration depleted lipids necessary for egg maturation and migration, those energetic losses might have been compensated for by other body tissues in order to spawn successfully the following spring (Idler & Clemens 1995; Hendry & Berg 1999). Fish can not survive loss of body components of this magnitude (Diana 1995). Although histological samples suggested that both trapped and non-trapped fish produced spawning capable gonads, trapped fish did not complete the fall migration back to the overwintering lake and, unlike non-trapped fish, remained undetected in further antenna surveys, suggesting that they died shortly after spawning or left the study area. This trade-off between survival and reproduction underscores the necessity for Arctic fish to capitalize on summer resource acquisition, for without sufficient resources, they might not meet

energetic thresholds, such as overwinter survival, spring reproduction and post-spawning survival. If the timing of dry zones disrupts fall migration, the direct effect might be increased adult mortality, but the indirect effect extends to a reduction of future fecundity by reducing iteroparity of the spawning population.

With accelerated climate change in the Arctic due to polar amplification of warming, the frequency and duration of aquatic habitat fragmentation in tundra streams will likely increase. If Arctic grayling populations repeatedly incur severe resource depletion, persistence of headwater populations becomes improbable due to changes in vital rates. I found that entrapment by dry zones not only affects death rates by increasing adult mortality, but indirectly affects birth rates by decreasing future spawning events for this iteroparous species. Iteroparity benefits Arctic grayling by providing multiple spawning opportunities in a highly variable environment, such that some cohorts experience favorable conditions for growth and survival in only some years (Deegan et al. 1999). Life history theory predicts a shift toward early maturation where adult mortality is high, as shown for Arctic grayling in central Norway (Haugen 2000). But, if strong selection also acts against repeated migration by adults, a shift from iteroparity toward greater and earlier reproductive investment might ensue. This type of life history trajectory with higher numbers of egg compensating for a shorter-lived population (i.e. McBride & Thurman (2003)) however, might not optimize overall survival of young in the environmentally variable Arctic. Nevertheless, the ramifications of entrapment for Arctic grayling likely include reduced population size combined with increased population isolation. Climate-induced aquatic habitat fragmentation, therefore, might increase extirpation risk for local Arctic grayling populations that rely on migration through drought prone river reaches.

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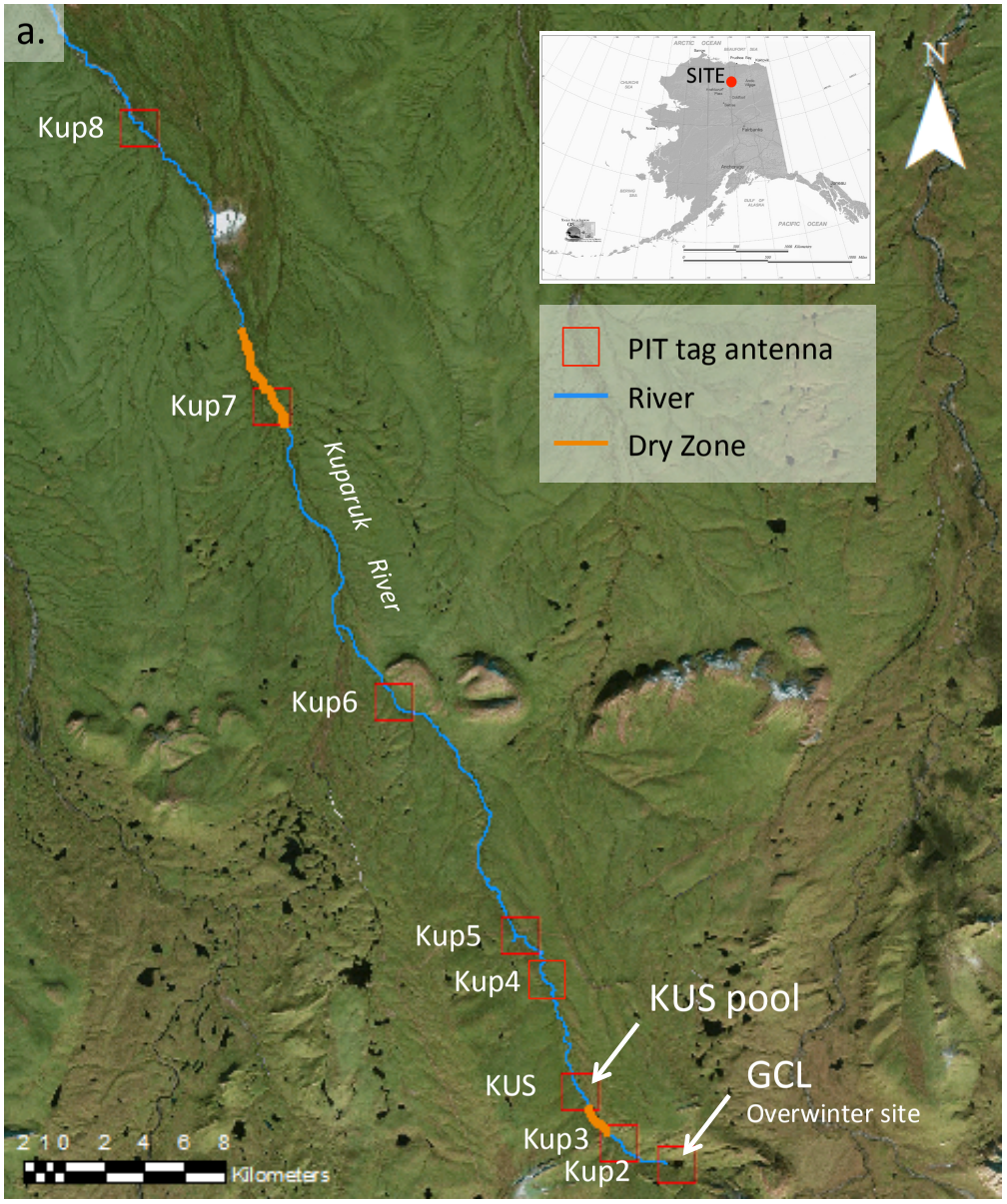

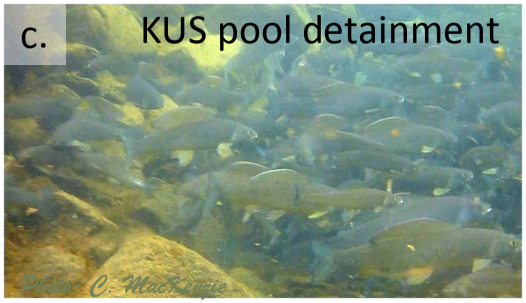

Figure 1. Location of study area (a.), indicating the Kuparuk River (blue lines), PIT tag antenna locations (red squares) and dry zones (orange lines); the Kup3 dry zone (b.); and fish detained in high density in the KUS pool below the Kup3 dry zone (c.). (Photos by A. Huryn (b.) and C. MacKenzie (b.))

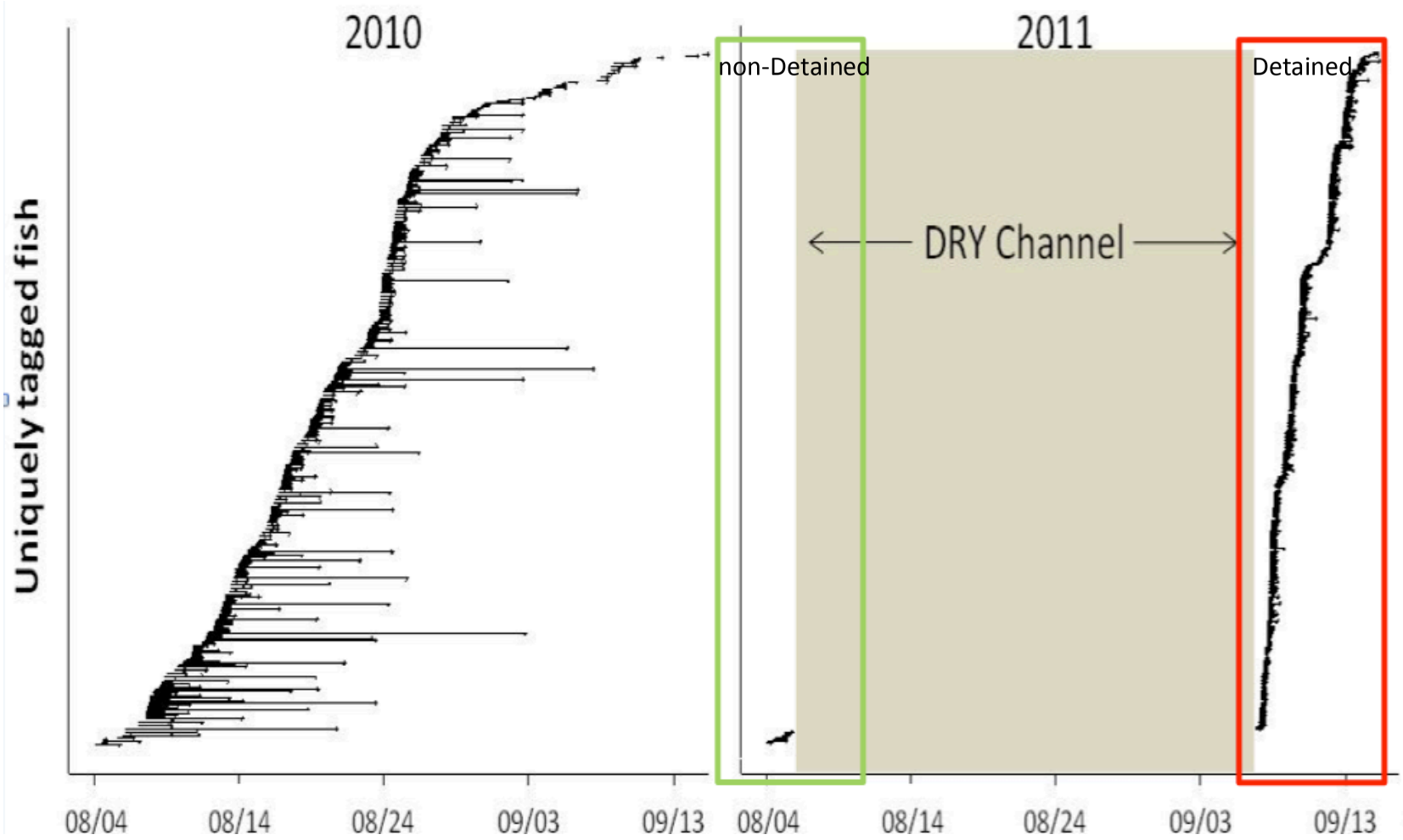

Figure 2. Fall movement patterns of PIT tagged individuals indicating last detection in the river (beginning of arrow) and first detection at the overwintering lake (arrow head) during an unobstructed year (2011) and an obstructed year (2011). In 2011 non-detained fish (green) migrated to the headwater lake early, while detained fish (red) remained in the river for the duration of the drought before migrating to the headwater lake.

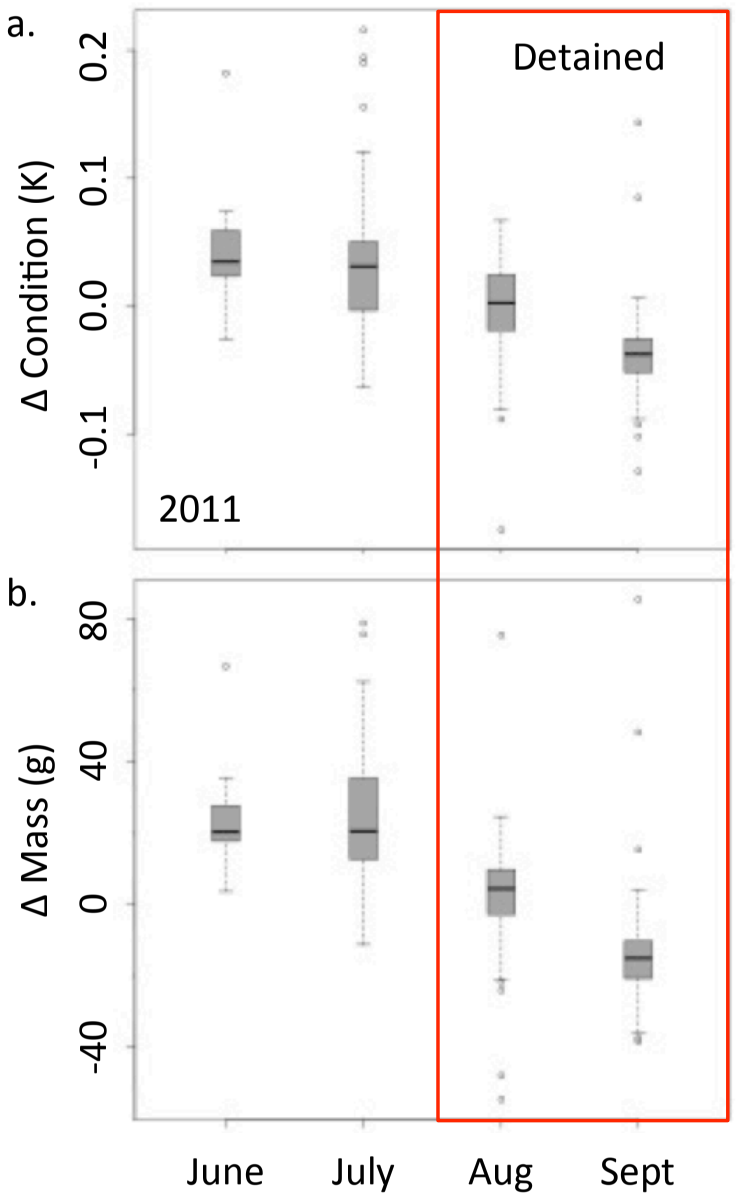

Figure 5. Change in condition (Fulton's K) (a.) and weight (g) (b.) of fish during the summer growing season and while detained in the KUS pool (highlighted in red).

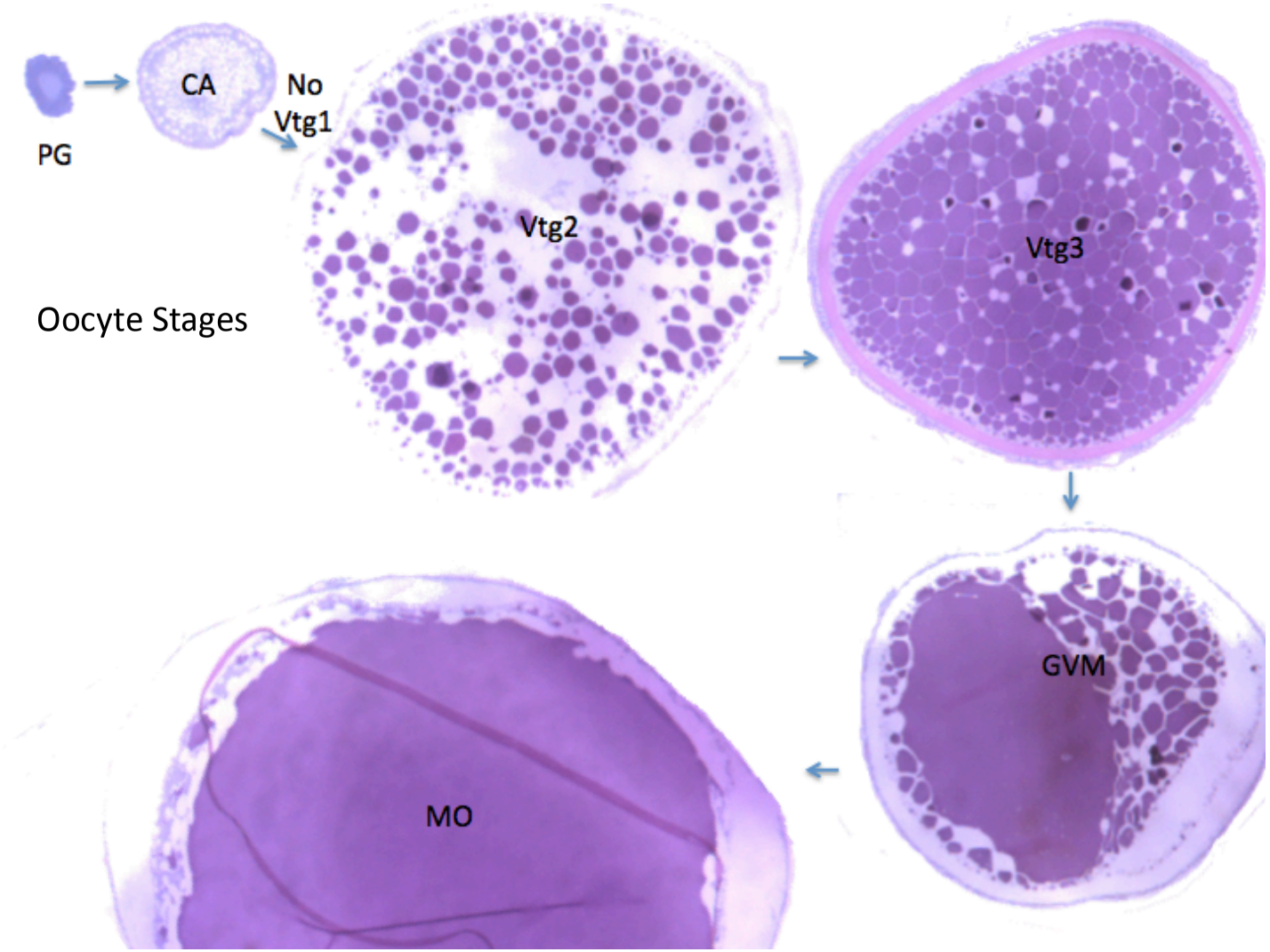

Figure 4. Oocyte stages. PG – primary growth; CA – cortical alveolar; Vtg1 - vitellogenesis stage 1 (none present); Vtg2 - vitellogenesis stage 2; Vtg3 - vitellogenesis stage 3; GVM – germinal vesicle migration (EVBD – early vesicle breakdown); MO – mature oocyte.

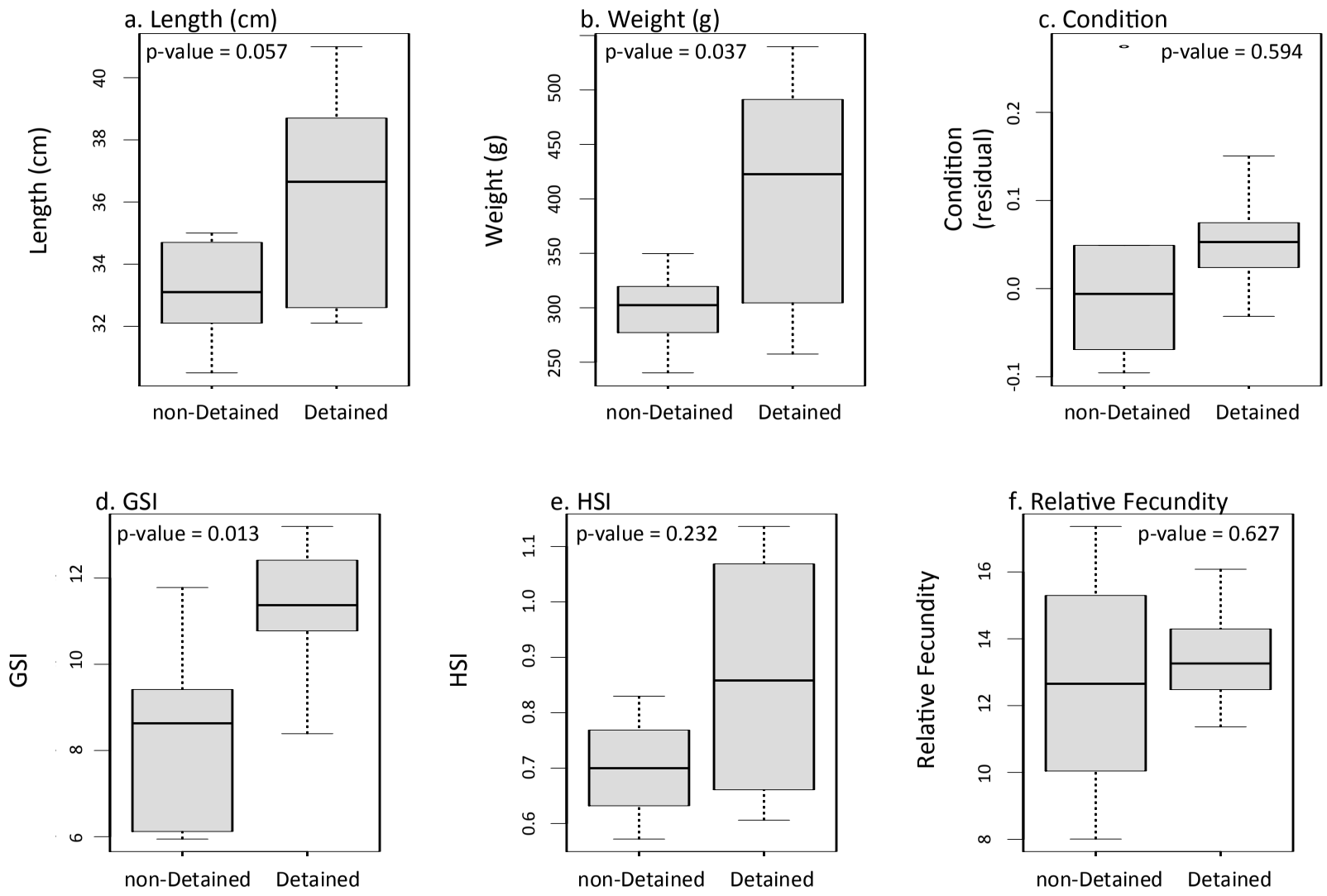

Figure 6. Differences in female post-overwintering length (cm) (a.), weight (gram) (b.), condition (c.), gonadosomatic index (GSI) (d.), hepatosomatic index (HSI) (e.), and relative fecundity (f.) between non-detained and detained individuals. Significance assessed using permutation tests on mean differences between groups.

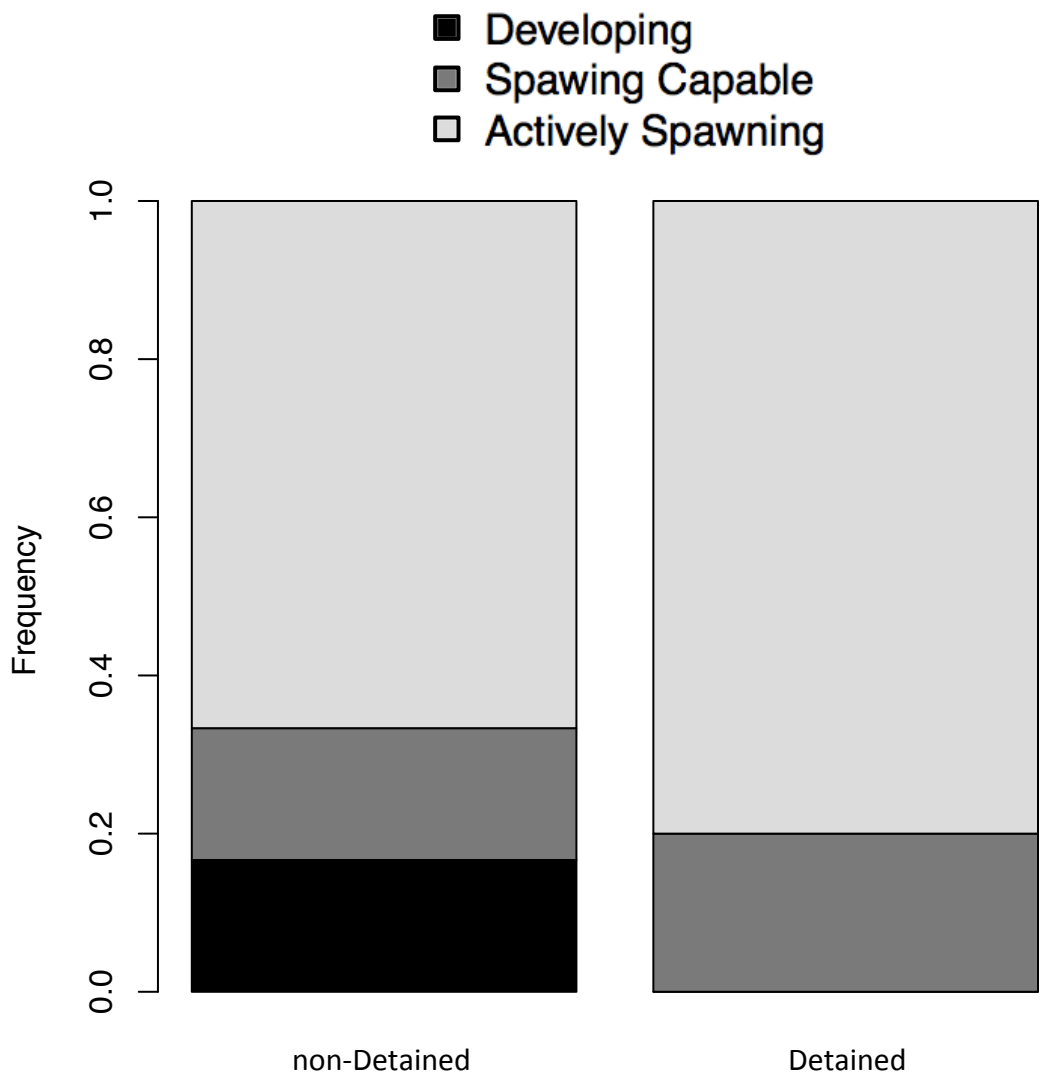

Figure 8. Ovary phases for non-detained and detained females.

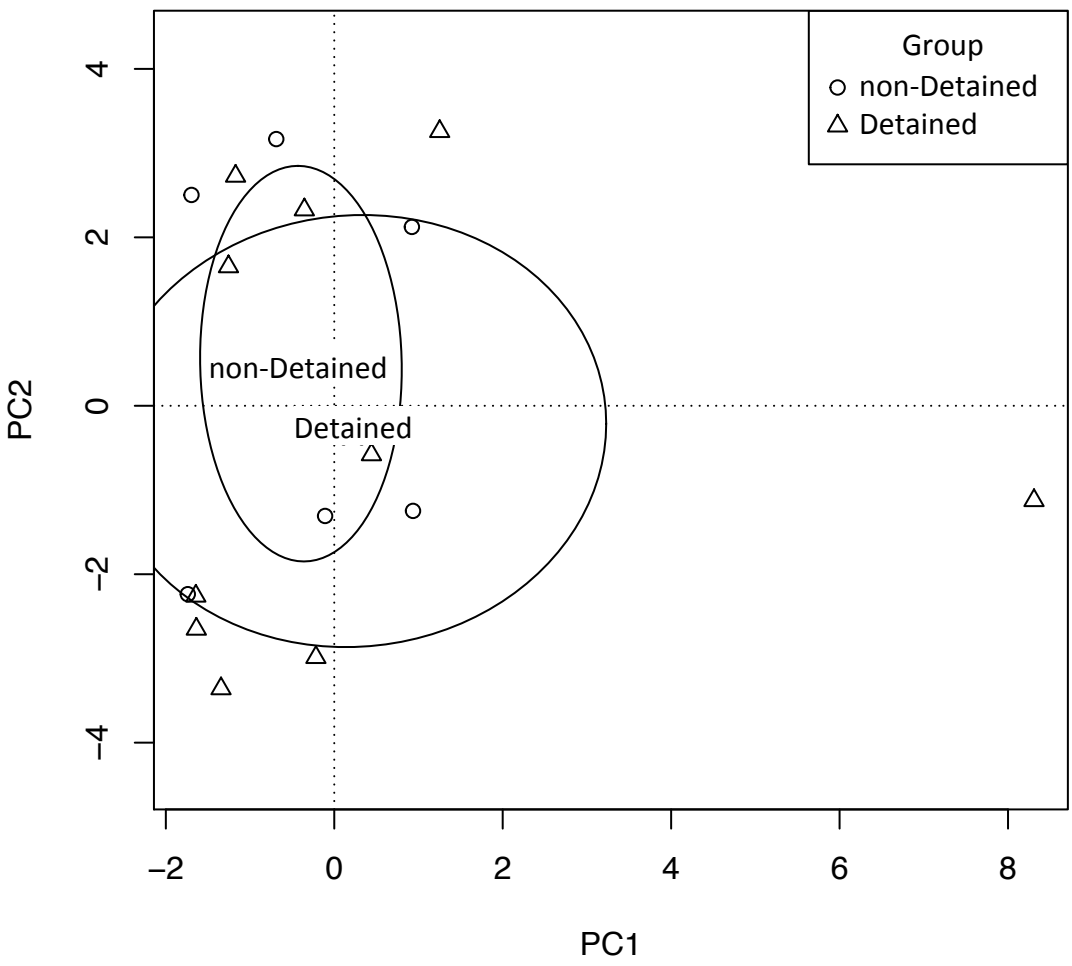

Figure 7. Redundancy analysis principal components 1 and 2, showing strongly overlapping ellipses (standard deviation) for the group factor (non-detained versus detained). None of the factors tested (length, weight, condition or group) explained significant variation in oocyte stage among individuals.

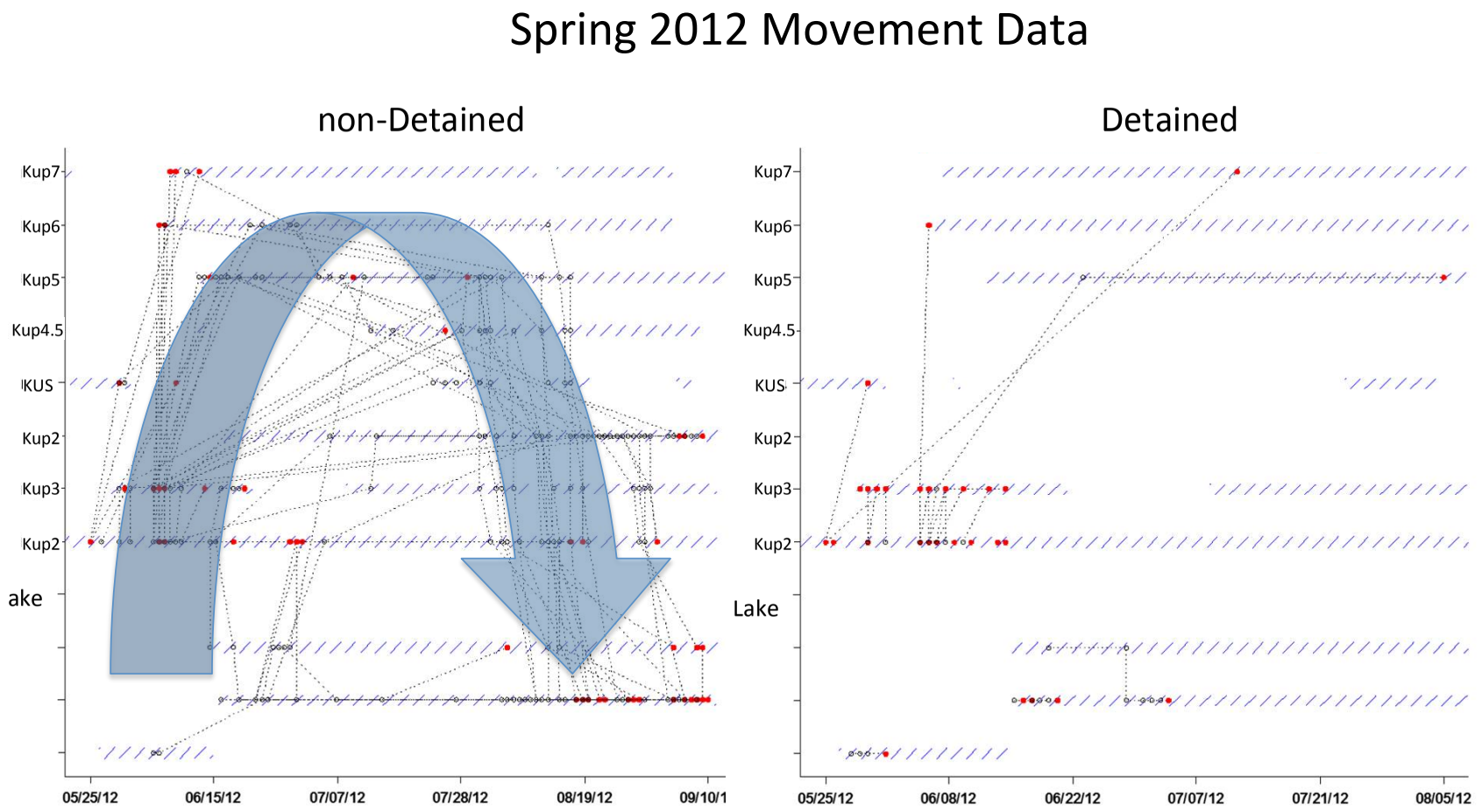

Figure 9. PIT-tag movement data from the headwater lake (Lake) downstream (Kup2 to Kup7) from May to September 2012 for non-detained and detained fish. Grey lines = fish movement paths, red dots = last detection, hash marks = functioning antenna.

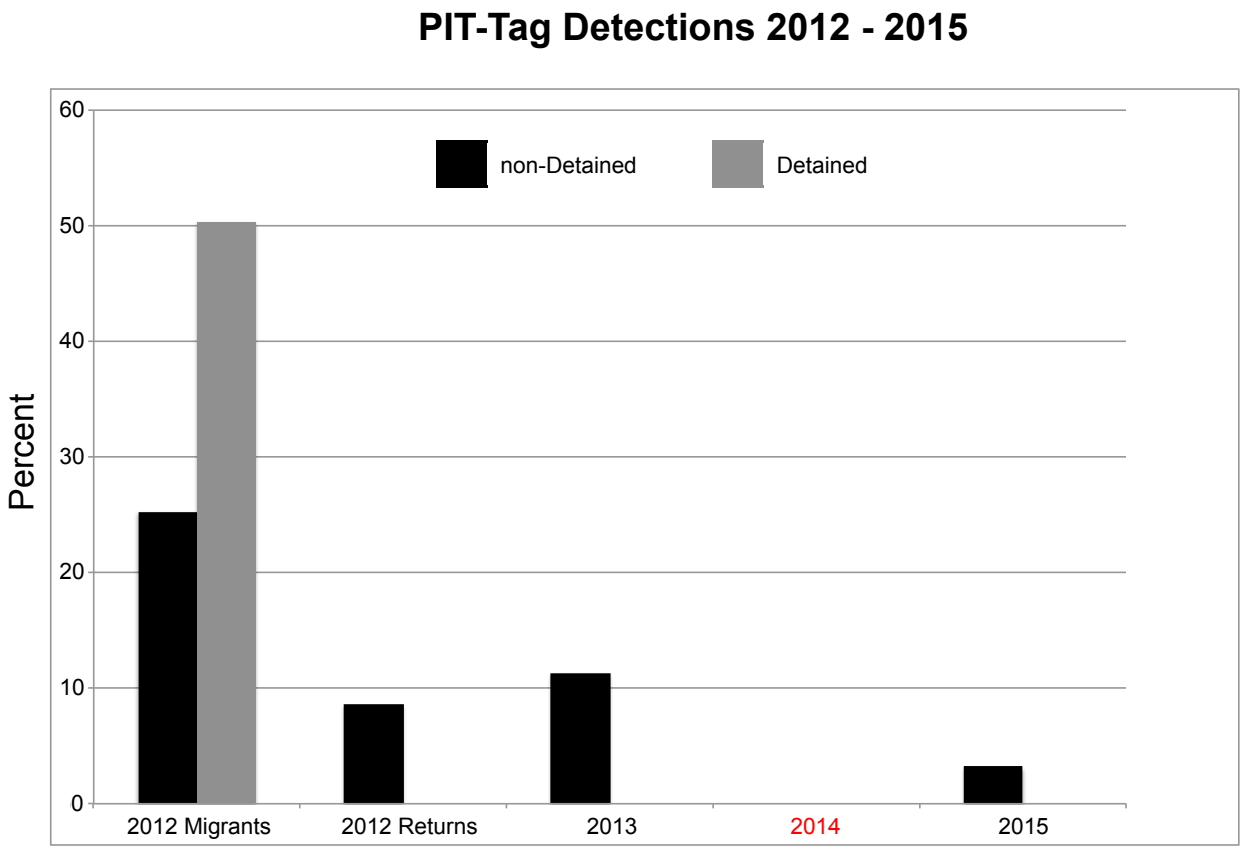

Figure 10. Percent of non-detained and detained PIT-tagged adults that migrated from the lake to the river in the spring of 2012 (2012 Migrants), percent of the 2012 spring migrants that returned to the lake in the fall of 2012 (2012 Returns), percent of non-detained and detained PIT-tagged adults that were detected in 2013 and in 2015. 2014 is missing data.

Table 1. Oocyte stages and identification descriptions (a) and ovary phases with diagnostic criterion (b). PG – primary growth; CN – chromatin nucleolar; PN – perinucleolar; CA – cortical alveolar; Vtg1 - vitellogenesis stage 1; Vtg1 - vitellogenesis stage 2; Vtg1 - vitellogenesis stage 3; GVM – germinal vesicle migration (EVBD – early vesicle breakdown); GVBD – germinal vesicle breakdown (LVBD – late vesicle breakdown); Hydr – hydration; POF – post ovulatory folicles; Atr – atresia.

a.

Oocyte Stage		Description
PG	CN	Smaller, less dense cytoplasm
	PN	Dense cytoplasm, nucleus w many nucleoli
CA		Chorion present - vesicles unfilled
Vtg1		Small filled yolk vesicles around perifery or nuclues
Vtg2		Filled vesicles present throughout cytoplasm
Vtg3		All vesicles full but not fused
GVM		Fusion of vesiles < 50% (EVBD)
GVBD		Fusion of vesiles >50% (LVBD)
Hydr		100% Fusion (SR)

b.

Phase	PG	CA	Vtg1	Vtg2	Vtg3	GVM	GVBD	POF	Atr
Immature	X								
Early Developing	X	X							
Developing	X	X	X	X		x			x
Spawning capable	X	X	X	X	X	x		X	x
Actively Spawning	X	X	X	X	X	X	X	X	x
Regressing	x	x	x	x				X	X
Regenerating	X							x	x

Table 2. Sample size (n), mean and standard deviation (stdev) for female and male Arctic grayling from non-detained and detained groups for length (cm), weight (g), condition (residual), hepatosomatic index (HSI), gonadosomatic index (GSI, females only), fecundity (number of mature or maturing oocytes per female), relative fecundity (number or mature or maturing oocytes per gram of female body weight).

	non-Detained						Detained					
	Female			Male			Female			Male		
	n	mean	stdev	n	mean	stdev	n	mean	stdev	n	mean	stdev
Length (cm)	6	33.1	±1.7	18	35.8	±2.6	10	36.1	±3.3	6	36.1	±2.6
Weight (g)	6	298.6	±37.3	18	361.6	±85.5	10	404.4	±104.4	6	373.6	±96.2
Condition (residual)	6	0.024	±0.134	18	-0.029	±0.035	10	0.051	±0.052	6	-0.023	±0.060
HSI	4	0.70	±0.11	13	0.82	±0.17	4	0.86	±0.25	4	1.00	±0.11
GSI	6	8.42	±2.18				10	11.25	±1.54			
Fecundity	4	3244	±441				9	4681	±1131			
Relative Fecundity	4	12.7	±3.8				9	13.4	±1.6			

Table 3. Ovary phases for non-detained and detained Arctic grayling as determined by stage, presence and abundance of oocytes. Oocyte stages: PG – primary growth; CN – chromatin nucleolar; PN – perinucleolar; CA – cortical alveolar; Vtg1 - vitellogenesis stage 1; Vtg1 - vitellogenesis stage 2; Vtg1 - vitellogenesis stage 3; GVM – germinal vesicle migration; GVBD – germinal vesicle breakdown; POF – post ovulatory folicles; Atr – Atresia.

Treatment	FishID	PG	CA	Vtg1	Vtg2	Vtg3	GVM	GVBD	POF	Atr	Ovary_Phase
non-Detained	W_114	17	18	0	0	0	7	13	0	0	Asp
	W_339	33	8	0	0	0	13	3	0	0	Asp
	W_342	10	5	0	13	0	2	0	0	1	D
	W_540	18	9	0	13	10	0	0	0	0	SpC
	W_762	5	11	0	0	0	1	14	0	0	Asp
	W_764	32	10	0	0	0	2	16	0	0	Asp
Detained	W_015	26	12	0	0	0	2	11	0	0	Asp
	W_117x	9	7	0	0	0	0	20	4	0	Asp
	W_123	19	12	3	6	9	0	0	0	0	SpC
	W_336	6	11	0	0	0	0	16	0	0	Asp
	W_338	12	11	0	14	6	0	0	0	0	SpC
	W_340	20	11	0	0	0	0	21	0	0	Asp
	W_343	11	9	0	1	1	14	1	0	0	Asp
	W_541	34	13	0	8	0	12	1	0	0	Asp
	W_766x	65	45	0	0	0	0	11	36	0	Asp
	W_768	10	4	0	0	0	0	16	0	0	Asp

Table 4. Number of Arctic grayling from non-detained and detained groups detected by PIT tag antennas.

	Group	
PIT Tag Antenna Detection	non-Detained	Detained
Entered Lake in 2011	961	286
Migrated to River in 2012	243	144
Returned to Lake in 2012	21	0
Detected in 2013	109	0
Detected in 2014	missing data	
Detected in 2015	31	0

Conclusions

Climate driven changes in hydrology that alter aquatic habitat connectivity on Alaska's North Slope (ACIA 2004; Bowden et al. 2008; Martin et al. 2009) will likely continue (Hinzman et al. 2005; Kane et al. 2004; Brosten et al. 2006; Zarnetske et al. 2008) with unknown consequences for species persistence. With predicted extinction of one out of six species under climate change scenarios (Urban 2015), understanding mechanisms driving species extinction might help mitigate losses in biodiversity due to rapidly changing environmental conditions. In the Arctic, increased river drying presents challenges to freshwater species by reducing habitat connectivity and movement of individuals among and within local populations. Because species persistence depends largely upon balances between gene flow, local adaptation and drift (Ovaskainen & Hanski 2004; Hanski et al. 2011), predictive models should highlight the importance of habitat connectivity. Throughout this dissertation, climate-induced habitat fragmentation by river drying appeared as a central theme in my investigation of Arctic grayling broad-scale population structure, fine-scale microgeographic differentiation, and local population vital rates.

In chapter one, I investigated factors influencing metapopulation structure of Arctic grayling on Alaska's North Slope. Using data from fifteen locations in the foothills of the Brooks Mountains and one location on the coastal plain, I discovered five distinct genetic clusters. River distance and dry river zones surfaced as significant factors explaining genetic differentiation among local populations. Asymmetrical gene flow among genetic clusters stemmed from small headwater populations in the Brooks Mountains to a large population located near the coastal plain and extending into the Itkillik watershed. Metapopulation structure in this system best represented a mainland-island metapopulation, but differed from the mainland-island model with regard to the direction of gene flow. With limited gene flow among genetic clusters, headwater populations might provide locally adapted genotypes to the mainland population through downstream dispersal of individuals. Thus, the mainland population might act as a "genetic

reservoir," which could replenish extirpated habitat patches so long as some degree of aquatic connectivity persists.

In chapter two, I tested for microgeographic neutral genetic differentiation and associations between an adult trait, migration distance, and neutral genetic differentiation within two genetically distinct populations. Neutral genetic differentiation can occur within dispersal range of a species when strong selection favors traits that reduce gene flow (Maan et al. 2004; Garcia de Leaniz et al. 2007; Richardson & Urban 2013). Due to the presence of drought-prone and drought-resistant river reaches, I predicted that within population genetic differentiation would correspond to adult migration distance, thereby segregating spawning activity. I found significant within-watershed genetic differentiation for larval Arctic grayling within both the Kuparuk and Oksrukuyik watersheds. Both watersheds consisted of distinct headwater and downstream populations that exhibited different distances moved and direction of movement. Variation in migration distance corresponded to fine-scale neutral genetic differentiation in the Kuparuk watershed. Spawning site fidelity, which might have evolved through selection or through site fidelity, might explain patterns of microgeographic differentiation and migration patterns for Arctic grayling in tundra streams with drought-prone and drought-resistant river reaches.

In chapter 3, I examined the effects of drought on vital rates and movement patterns of Arctic grayling in the Kuparuk River. Because environmental conditions leading up to spawning can influence spawning capability of fish (Lubzens et al. 2010), I predicted that drought would negatively affect Arctic grayling fecundity and survival. I found that detainment due to drought substantially decreased fish mass and condition during the drought. However, I found no significant differences in body condition, rates of oocyte atresia, or spawning movement patterns between detained and non-detained fish moving out of the overwintering location the following spring (2012). Nevertheless, I found significant differences in fall movement patterns and adult survival between detained and non-detained individuals. Detainment by drought affected local population vital rates by increasing adult deaths and decreasing future fecundity for this iteroparous species.

Although Arctic grayling is prolific across most of its Holarctic distribution, population extirpations (i.e. the Michigan population), declines (i.e. Montana and Williston River, British Columbia populations), and range contractions (i.e. the Canadian Athabasca, Peace and Hay River populations) reflect the susceptibility of this species to local extirpation due to anthropogenic factors, including habitat fragmentation, habitat destruction and climate change (US Dept. Interior 2010). Summarizing findings from my research, I found that reduced connectivity due to river drying caused increased population structure by reducing gene flow among local populations on a broad-spatial scale. Within local populations, I found that river drying and entrapment by dry zones altered local population vital rates and movement patterns, which might exert strong selection on local populations for particular migratory phenotypes. Thus, under future climate change, I predict that increased river drying could further isolate local headwater populations and reduce population size, which intrinsically leads to increased extirpation risk, particularly for local headwater populations. The large coastal population, however, retains potential to harbor genetic diversity, provided local adaptation and gene flow from the headwaters to the coastal plain persists. Additionally, individuals from the coastal population might replenish extirpated habitat patches, given adequate aquatic habitat connectivity, thereby providing resilience to the North Slope Arctic grayling metapopulation under future climate change conditions. My research on Arctic grayling underscores the significance of maintaining habitat connectivity for metapopulation persistence and the importance of including connectivity in conservation and management models to help mitigate the effects of climate change on species extinctions.

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